The Journal of
CLINICAL ENDOCRINOLOGY
AND METABOLISM

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Studies on Fertility

THE PROCEEDINGS OF THE SOCIETY FOR THE STUDY OF FERTILITY

The Society for the Study of Fertility had its origin in an informal meeting of a few scientists interested in seminology. This was held in 1944; since then the Society has met annually and has grown in numbers considerably. The membership is representative of many fields of study, and includes among others research workers in zoology, anatomy, physiology, endocrinology, clinical medicine, agricultural science, veterinary practice and sociology.

In the past the Proceedings of the Society have been published annually in journal form. Material of such importance, however, was felt to be worthy of a more permanent record; in future, therefore, the Society's policy will be to present as a complete book each year, or more frequently if desirable, a selection of the papers read at the annual Conference together with other papers of high standing.

This policy will be widely welcomed by all those whose work involves problems of fertility and who cannot afford to neglect the latest results of research in this important field. The contents of Volume VI, which will be published in March, are given below; a detailed prospectus, listing the contents of all back numbers, is available on request.

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London Conference, 1954

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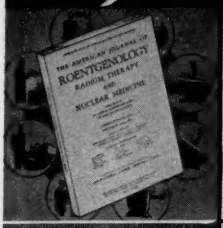

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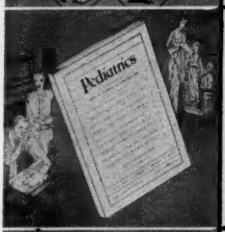

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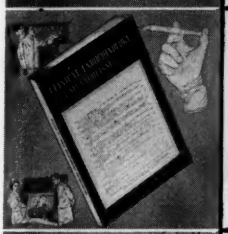

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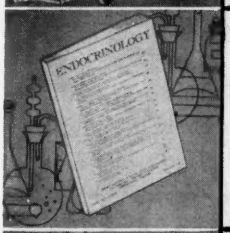

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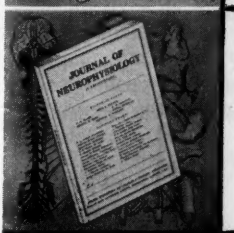

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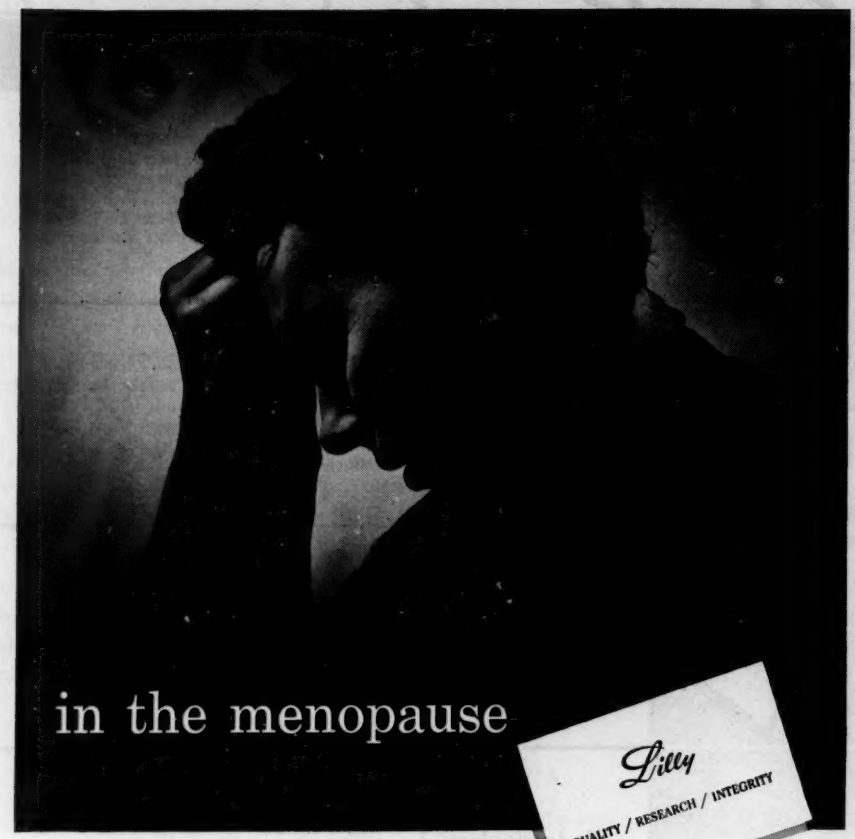

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THE EFFECTIVENESS OF TRIIODOTHYRONINE OR THYROXINE ADMINISTERED ORALLY IN THE TREATMENT OF MYXEDEMA*†

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THE introduction of the use of whole thyroid gland in the treatment of myxedema in 1891 (1, 2) pioneered the development of specific hormonal replacement therapy. Thyroxine, believed to be the effective principle of the thyroid gland, was isolated in 1915 by Kendall (3). Its chemical structure was determined and its synthesis was accomplished in 1927 by Harington and Barger (4). It has been clearly demonstrated that identical metabolic effects result from the administration of desiccated thyroid and synthetically prepared thyroxine. However, the latter was thought to be ineffective when administered orally to patients with myxedema due to poor absorption from the gastrointestinal tract (5). The numerous difficulties inherent in the chemical synthesis of thyroxine permitted only limited quantities for experimental studies and impeded more extensive investigations on the use of thyroxine by mouth. Desiccated thyroid has thus remained for more than sixty years the agent of choice in the treatment of myxedema.

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† An abstract of this paper has previously appeared in *J. Clin. Invest.* **33**: 966, 1954.

‡ United States Public Health Service Fellow in Medicine, 1953–1954.

The efficacy of administering thyroxine orally was re-evaluated in 1933 by Thompson and his co-workers (6) who demonstrated that the monosodium and disodium salts of thyroxine, probably due to their increased solubility, had greater potency than the acid form of the hormone when given by mouth. These sodium salts of thyroxine were demonstrated to be of therapeutic value in myxedema; however, since this form of therapy was expensive and offered no therapeutic advantage over desiccated thyroid, it failed to achieve clinical acceptance.

The development in 1949 of a commercially feasible synthesis of *l*-thyroxine (7), the naturally occurring isomer, made large quantities of this compound available for study. Assay of *l*-thyroxine in animals showed that it possessed twice the calorigenic potency of the racemic compound, *dl*-thyroxine (8–11); this ratio also appears to apply to its calorigenic effects in athyreotic patients (12). Recent studies of the monosodium salt of *l*-thyroxine in human subjects (13–19) have demonstrated its uniform effectiveness in the treatment of myxedema. Doses of 0.2 to 0.5 mg. daily by mouth produce the same metabolic effects as 128 to 192 mg. (2 to 3 grains) of desiccated thyroid. Sodium *l*-thyroxine administered orally has thus been shown to be efficacious in the treatment of myxedema.

The discovery of 3,5,3′-*l*-triiodothyronine has led to a re-evaluation of our concepts concerning the thyroid hormone. This analogue of thyroxine has been isolated from human plasma following I^{131} administration (20); it has also been identified in plasma following the administration of I^{131}-labeled thyroxine to mice (21) and to an athyreotic human subject (22). These findings have led to the postulation by Gross and Pitt-Rivers (23) that thyroxine is deiodinated in the peripheral tissues to triiodothyronine, the effective form of the thyroid hormone. The remarkable potency of *l*-triiodothyronine further supports this postulation. It is approximately five times as effective as *l*-thyroxine in preventing thiouracil-induced goiters in rats (23–28) and is more effective than *l*-thyroxine in elevating the oxygen consumption of mice and rats (25–27, 29–33). When administered parenterally in single or multiple doses to patients with myxedema, *l*-triiodothyronine produces three to five times the calorigenic effect of *l*-thyroxine (12, 34, 35). It has been established that this compound produces all the metabolic alterations ascribed to the thyroid hormone; it differs from desiccated thyroid and thyroxine only in that its effects are produced more rapidly and by smaller doses (12). Moreover, the effects of triiodothyronine do not appear to persist as long as those of thyroxine, perhaps due to a difference in the manner in which triiodothyronine is metabolized.

The purpose of this study was to assay the effects of *l*-triiodothyronine, *dl*-triiodothyronine and *l*-thyroxine administered daily by the oral route,

in order to determine the relative effectiveness of these substances in maintaining euthyroidism in patients with myxedema.

METHODS

Six patients were selected for study. Each exhibited the classic signs, symptoms and metabolic derangements of myxedema, and the results of a radioactive iodine tracer test and the level of serum protein-bound iodine further substantiated this diagnosis. All were treated on an out-patient basis.

Serial basal metabolic rates were determined during the entire study by a single trained observer. Each recording of the basal metabolic rate represents the average of the results of duplicate determinations.

Assays of serum protein-bound iodine were performed by the Bio-Science Laboratories, Beverly Hills, California, using a modification of the method described by Barker (36). Serum cholesterol determinations were made by the clinical laboratory of this hospital (37). All blood samples were drawn with the patient in the fasting state. When patients were receiving therapy, blood samples were drawn at various intervals and on each occasion were taken twenty-four hours following the previous dose of medication.

The *l*-triiodothyronine was administered in tablets, each containing 35 micrograms of 3,5,3′-*l*-triiodothyronine.[1] Thyroxine was given in tablets, each containing 100 micrograms of monosodium *l*-thyroxine.[1] The *dl*-triiodothyronine was administered in gelatin capsules, each containing 50 micrograms of 3,5,3′-*dl*-triiodothyronine hydrochloride.[2] Patient J. C. (Fig. 4) received *dl*-triiodothyronine in the form of the free amino acid during the initial trial of therapy; subsequently, this patient was given *dl*-triiodothyronine hydrochloride. Unless otherwise noted, the *dl*-triiodothyronine referred to in this study is always *dl*-triiodothyronine *hydrochloride*.

The effectiveness of a specified dose of a compound in maintaining euthyroidism was determined in each patient studied, sufficient time being allowed to permit the compound to exert its full metabolic effect. At varying intervals, either the dose or the compound was changed without lapse in therapy. Each compound was administered once daily by mouth.

RESULTS

Effects on clinical status. The daily oral administration of 70 to 105 micrograms of *l*-triiodothyronine or 150 to 200 micrograms of *dl*-triiodothyronine to patients with myxedema (Figs. 1, 2, 3 and 5) resulted in a return to clinical euthyroidism within ten to fourteen days. There was rapid and complete disappearance of all signs and symptoms of hypothyroidism during this period of time without any distressing or toxic effects being noted. Each patient lost 6 to 10 pounds in weight during the first two weeks of treatment with triiodothyronine and remained euthyroid for the duration of this therapy.

In Patient J. C. (Fig. 4), the initial administration of 200 micrograms

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[1] The 3,5,3′-*l*-triiodothyronine and sodium *l*-thyroxine were kindly supplied by Dr. A. E. Heming of Smith, Kline and French Laboratories, Philadelphia, Pa.

[2] The 3,5,3′-*dl*-triiodothyronine was kindly supplied by Dr. E. L. Sevringhaus of Hoffmann-La Roche, Inc., Nutley, N. J.

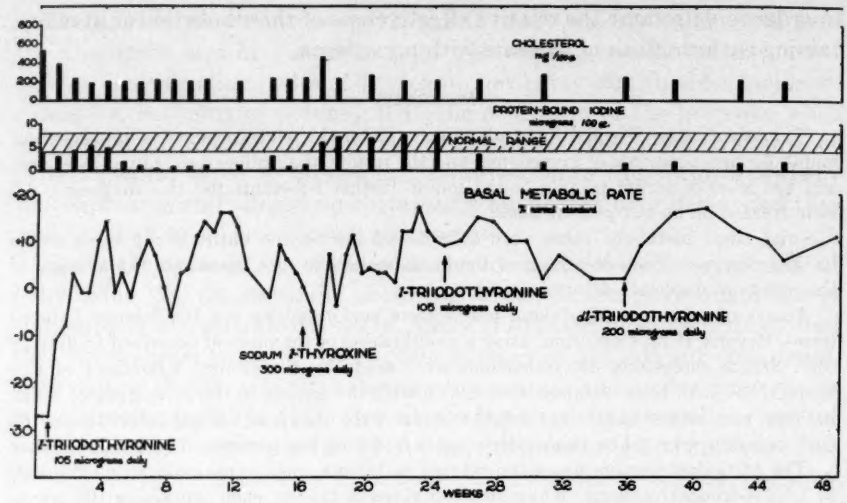

FIG. 1. Comparative effects on the levels of the basal metabolic rate, serum cholesterol and serum protein-bound iodine produced by the daily oral administration of *l*-triiodothyronine, *dl*-triiodothyronine (hydrochloride) or sodium *l*-thyroxine to a 46-year-old woman with postoperative myxedema. In this, as well as in subsequent figures, each arrow indicates the day on which either daily medication was initiated or the indicated change in daily medication was made.

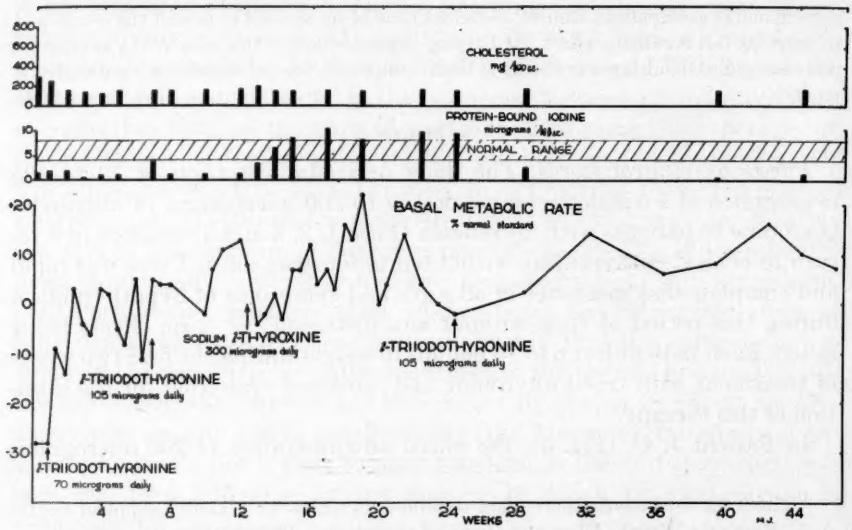

FIG. 2. Comparative effects on the levels of the basal metabolic rate, serum cholesterol and serum protein-bound iodine produced by the daily oral administration of *l*-triiodothyronine or sodium *l*-thyroxine to a 53-year-old woman with postoperative myxedema.

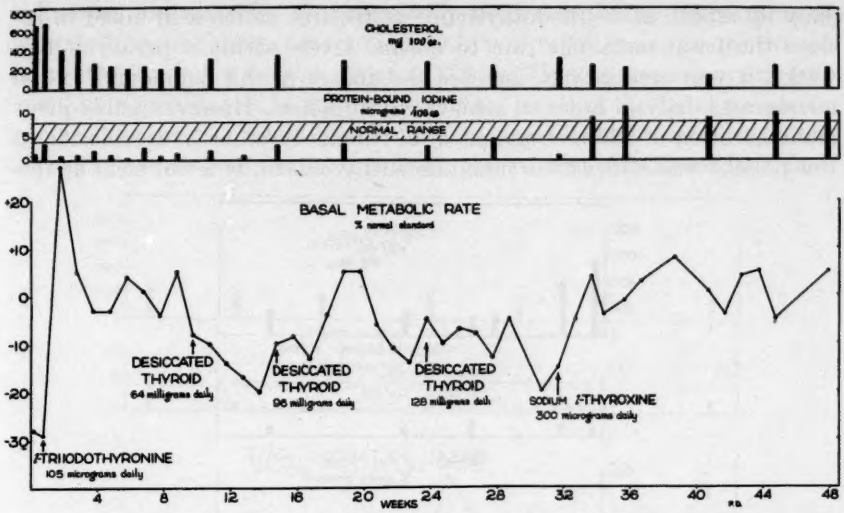

FIG. 3. Comparative effects on the levels of the basal metabolic rate, serum cholesterol and serum protein-bound iodine produced by the daily oral administration of *l*-triiodothyronine, desiccated thyroid or sodium *l*-thyroxine to a 42-year-old woman with postoperative myxedema.

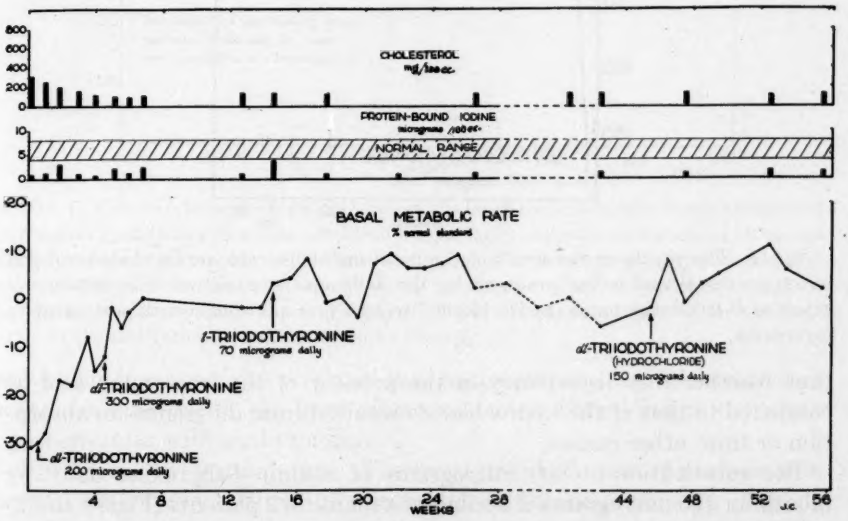

FIG. 4. Comparative effects on the levels of the basal metabolic rate, serum cholesterol and serum protein-bound iodine produced by the daily oral administration of *dl*-triiodothyronine (free amino-acid form), *l*-triiodothyronine or *dl*-triiodothyronine (hydrochloride) to a 24-year-old woman with spontaneous myxedema.

daily by mouth of *dl*-triiodothyronine as the free amino acid failed to restore the basal metabolic rate to normal levels within a period of five weeks; it was necessary to increase the dosage of this compound to 300 micrograms daily in order to achieve euthyroidism. However, subsequent administration of 150 micrograms of *dl*-triiodothyronine hydrochloride to this patient was sufficient to maintain euthyroidism. It is not clear at this

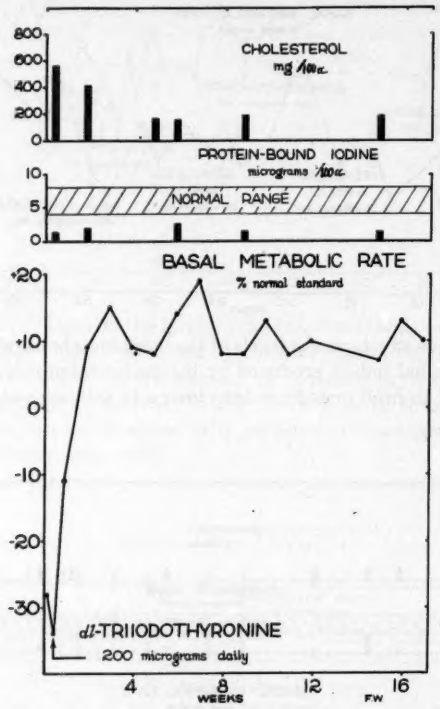

FIG. 5. The effects on the levels of the basal metabolic rate, serum cholesterol and serum protein-bound iodine produced by the daily oral administration of 200 micrograms of *dl*-triiodothyronine (hydrochloride) to a 58-year-old woman with postoperative myxedema.

time whether this discrepancy in the potency of the free amino acid as compared to that of the hydrochloride resulted from differences in absorption or from other causes.

The substitution of 300 micrograms of sodium *l*-thyroxine daily by mouth for 105 micrograms of *l*-triiodothyronine in 2 patients (Figs. 1 and 2) produced no appreciable change in their clinical or metabolic status. These dosages appeared to be equivalent in their effects on the maintenance of the basal metabolic rate at euthyroid levels.

Another patient (Fig. 3), successfully treated with 105 micrograms of *l*-triiodothyronine daily, became mildly hypothyroid upon substitution of desiccated thyroid in daily doses up to 128 mg. Hypothyroidism was manifested by a gain in weight and by subnormal levels of the basal metabolic rate and the serum protein-bound iodine. Upon the administration of 300 micrograms of sodium *l*-thyroxine daily, this patient manifested increased

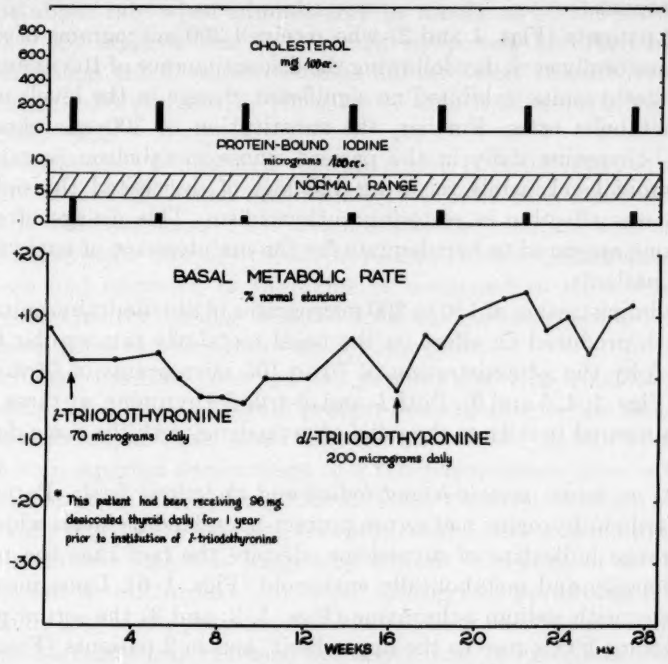

FIG. 6. Comparative effects on the levels of the basal metabolic rate, serum cholesterol and serum protein-bound iodine produced by the daily oral administration of 70 micrograms of *l*-triiodothyronine or 200 micrograms of *dl*-triiodothyronine (hydrochloride) to a 43-year-old woman with spontaneous myxedema. This patient had remained euthyroid when receiving daily oral doses of 96 mg. of desiccated thyroid for one year prior to the institution of triiodothyronine therapy.

well being, loss in weight, return of the basal metabolic rate to normal, and a concomitant rise in the level of serum protein-bound iodine to values commensurate with euthyroidism.

Effects on basal metabolic rate. The basal metabolic rate of each patient treated initially with 70 to 105 micrograms of *l*-triiodothyronine (Figs. 1, 2 and 3) returned to euthyroid levels within ten to fourteen days and remained there for the duration of this therapy. Similarly, the patient who had been maintained for one year prior to the present study on 96 mg. of

desiccated thyroid daily (Fig. 6) remained euthyroid for the four-month period following substitution of 70 micrograms of *l*-triiodothyronine daily by mouth. One patient (Fig. 3), mentioned previously, who remained completely euthyroid while receiving 105 micrograms of *l*-triiodothyronine daily by mouth, mainifested a decrease of her basal metabolic rate when doses up to 128 mg. of desiccated thyroid were substituted for the *l*-triiodothyronine.

The 2 patients (Figs. 1 and 2) who received 300 micrograms of sodium *l*-thyroxine orally each day following the discontinuance of 105 micrograms of *l*-triiodothyronine exhibited no significant change in the levels of their basal metabolic rates. Further, the substitution of 300 micrograms of sodium *l*-thyroxine daily in the patient whose metabolism remained at hypothyroid levels while receiving 128 mg. of desiccated thyroid daily (Fig. 3) was effective in restoring euthyroidism. This dosage of sodium *l*-thyroxine appeared to be adequate for the maintenance of euthyroidism in these patients.

The administration of 150 to 200 micrograms of *dl*-triiodothyronine daily by mouth produced an effect on the basal metabolic rate similar to that produced by the administration of 70 to 105 micrograms of *l*-triiodothyronine (Figs. 1, 4, 5 and 6). Both *l*- and *dl*-triiodothyronine, at these dosage levels, appeared to initiate the relief of myxedema with the same degree of rapidity.

Effects on serum protein-bound iodine and cholesterol levels. Patients receiving triiodothyronine had serum protein-bound iodine levels which were in the range indicative of myxedema, despite the fact that the patients were clinically and metabolically euthyroid (Figs. 1–6). Upon institution of therapy with sodium *l*-thyroxine (Figs. 1, 2, and 3) the serum protein-bound iodine levels rose to the upper limit, and in 2 patients (Figs. 2 and 3) above the upper limit generally accepted as normal.

The serum cholesterol level of each patient treated initially with triiodothyronine decreased to normal values as the patient achieved euthyroidism (Figs. 1–5). There was no observed difference in the degree or rate of change in cholesterol values following the administration of calorigenically equivalent doses of either *l*- or *dl*-triiodothyronine.

DISCUSSION

The results of this study indicate that triiodothyronine is an effective oral medication in myxedema in doses of 70 to 105 micrograms daily for the *l*-isomer and 150 to 200 micrograms daily for the racemic compound, *dl*-triiodothyronine. For sodium *l*-thyroxine, a dose of 300 micrograms daily was required to achieve the same effect. Unpublished observations in this laboratory on 3 other patients with myxedema indicate that doses smaller than the aforementioned may partially relieve myxedema but are

insufficient to maintain euthyroidism. Thus, *l*-triiodothyronine exhibits approximately three to four times the calorigenic effect of *l*-thyroxine. The racemic compound, *dl*-triiodothyronine, possesses approximately 50 per cent the calorigenic activity of *l*-triiodothyronine, suggesting (but by no means establishing) that *d*-triiodothyronine is inactive by mouth.

The observation that *l*-triiodothyronine produces the same response in basal metabolic rate when administered by mouth as by the intravenous route (34, 35) suggests that it is almost completely absorbed from the gastro-intestinal tract. Animal studies indicate that triiodothyronine is approximately 86 per cent as effective by mouth as when administered parenterally (23).

If the hypothesis is accepted that thyroxine is deiodinated to triiodothyronine *in vivo* (23), then theoretically, the total calorigenic effect following the administration of *l*-thyroxine should be identical with that following an equimolar dose of *l*-triiodothyronine. However, differences in the absorption and excretion of thyroxine as compared to triiodothyronine may account for the finding that the total calorigenic effect of *l*-triiodothyronine is three to four times that of *l*-thyroxine.

It previously has been observed that patients receiving triiodothyronine parenterally have levels of serum protein-bound iodine in the range indicating myxedema, at times when they are euthyroid (12, 34, 38–40). The results herein reported demonstrate that this phenomenon likewise follows oral administration of triiodothyronine. Thus, the level of serum protein-bound iodine in patients receiving triiodothyronine is clearly not indicative of their metabolic status as judged by current standards of the normal range for this determination (4.0 to 8.0 micrograms per 100 ml.). On the other hand, athyreotic patients receiving sodium *l*-thyroxine during the course of this study had values at the upper limit or exceeding the upper limit of normal, at times when otherwise they were euthyroid; a similar observation has been previously reported (16). In athyreotic patients receiving desiccated thyroid the level of serum protein-bound iodine has been reported to reflect their metabolic status (41), although recently the value of this determination in such patients has been questioned (16, 19).

The low level of the serum protein-bound iodine following the administration of triiodothyronine does not appear to result from failure of this compound to be bound by plasma proteins. It has been reported that triiodothyronine associates in loose combination with the plasma proteins (42), probably the alpha globulins; its low level in the plasma may therefore result from its rapid disappearance into tissue spaces (34). Although triiodothyronine has been demonstrated in the plasma of euthyroid and hyperthyroid subjects (19), it appears to be present in only minute quantities. Indeed, failure to isolate triiodothyronine from plasma has been reported (43).

Triiodothyronine acts rapidly in small doses to relieve myxedema; a single subcutaneous injection of 1.0 mg. of *l*-triiodothyronine produces clinical and metabolic changes in four hours and maximum effects in three to four days (12). However, this seemingly advantageous property of acting rapidly to relieve myxedema may be undesirable in some patients; for example, it may be hazardous in patients with myxedema and associated heart disease (44) or in patients with pituitary myxedema (45). Triiodothyronine, as well as large doses of thyroxine or desiccated thyroid, should therefore be employed with caution in initiating therapy in such patients.

Triiodothyronine is an effective therapeutic agent in myxedema; like thyroxine, it has the advantage of being a chemically pure compound not requiring standardization. However, triiodothyronine possesses no qualitative therapeutic advantage over desiccated thyroid or sodium *l*-thyroxine.

SUMMARY

The administration of triiodothyronine or thyroxine orally to patients with myxedema affords a uniformly effective form of thyroid hormone therapy. The daily oral maintenance dose of *l*-triiodothyronine is of the magnitude of 70 to 105 micrograms and of *dl*-triiodothyronine, 150 to 200 micrograms. Sodium *l*-thyroxine in daily oral doses of 300 micrograms produces comparable clinical and metabolic effects. Thus, *l*-triiodothyronine has three to four times the calorigenic effect of *l*-thyroxine. Further, *dl*-triiodothyronine has approximately 50 per cent the calorigenic activity of *l*-triiodothyronine, suggesting that *d*-triiodothyronine is inactive by mouth.

The level of the serum protein-bound iodine in athyreotic patients maintained in euthyroidism by daily administration of triiodothyronine remains in the range found in myxedema. Accordingly, this determination is not indicative of the metabolic status of these patients, as judged by current standards of the normal values for this determination.

The clinical and metabolic properties of triiodothyronine are qualitatively identical with those of thyroxine and desiccated thyroid. Like thyroxine, triiodothyronine is a chemically pure synthetic compound, obviating standardization.

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EFFECT OF TESTOSTERONE PROPIONATE ON THE PROSTATE GLAND OF PATIENTS OVER 45*

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THE observations which led to the conclusion that testosterone propionate has an aggravating effect on existing carcinoma of the prostate (1) raise the question as to whether or not testosterone propionate in doses used for the treatment of the male climacterium and angina pectoris is a factor in initiating carcinoma or activating latent carcinoma of the prostate gland.

In view of the seriousness of such a possibility, 100 adult males who had previously been exposed to testosterone therapy over periods varying from several months to several years were examined for evidence of carcinoma of the prostate or other pathologic changes of the gland. These men formed part of a larger group which had been studied by one of us (M.L.) several years previously in relation to the effect of testosterone propionate on angina pectoris or the male climacterium (2, 3, 4). Prior to treatment, each man had been examined to rule out existing neoplasm, and only those found by digital examination to be free of such growths had been accepted for the previous study. Testosterone propionate had then been administered to these patients intramuscularly in doses of 25 to 75 mg. per week for periods varying from three months to four years (Tables 1 and 2). In many of those who were observed and treated for several years, testosterone propionate was discontinued, sometimes for as long as six months. Treatment with testosterone propionate had been terminated between one and seven years before follow-up examinations were made for the purpose of this study.

All patients included in the group evaluated for the effect of the hormone on the prostate were 45 or more years of age. As controls, 100 adult males of comparable age were chosen at random from among men who had no

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TABLE 1. PROSTATIC ABNORMALITIES ACCORDING TO LENGTH OF TREATMENT WITH TESTOSTERONE PROPIONATE

Months of treatment	Total No. of patients	No. of abnormalities		No. of patients with one or both abnormalities
		Hypertrophy	Palpable irregularities	
3–5	21	5	3	7
6–11	23	9	2	9
12–17	24	4	1	4
18–23	11	3	2	5
24 & over	21	6	3	7
Total	*100*	*27*	*11*	*32*

genito-urinary symptoms, and who had never been treated with testosterone propionate (Table 3). Repeated examinations were made of those patients in the treated group in whom palpable irregularities of the prostate gland had developed. Serum acid phosphatase levels were not routinely determined. However, in those treated patients who showed palpable irregularities, these levels were determined and found to be normal.

Among the 100 treated patients, palpable irregularities of the prostate had developed in 11. With 1 exception, these lesions proved to be inflammatory in nature. In 10 of the 11 patients varying amounts of pus were found in the prostatic fluid. In this study "palpable irregularity" is defined as any palpable area of prostatic induration of a consistency greater than that of a normal prostate, yet less than that of the extreme hardness characteristic of prostatic malignancy. Examination of the eleventh pa-

TABLE 2. PROSTATIC ABNORMALITIES ACCORDING TO DOSAGE OF TESTOSTERONE PROPIONATE

Dosage (mg.)	Total No. of patients	No. of abnormalities		No. of patients with one or both abnormalities
		Hypertrophy	Palpable irregularities	
350– 500	9	2	2	4
500–1,000	41	14	3	14
1,000–1,500	27	3	2	3
1,500–2,000	15	5	3	7
2,000–2,500	4	1	1	2
2,500 & over	4	2	0	2
Total	*100*	*27*	*11*	*32*

TABLE 3. PROSTATIC ABNORMALITIES ACCORDING TO AGE OF THE PATIENT

Age	*No. of patients*		*Treated*			*Control*		
			No. of abnormalities		No. of patients with one or both abnormalities	No. of abnormalities		No. of patients with one or both abnormalities
	Treated	Control	Hypertrophy	Palpable irregularities		Hypertrophy	Palpable irregularities	
45–50	23	18	6	3	8	3	1	3
51–60	40	49	13	5	15	16	6	18
61–70	30	24	5	2	7	10	3	11
70 & over	7	9	3	1	2	5	3	7
Total	*100*	*100*	*27*	*11*	*32*	*34*	*13*	*39*

tient, an 80-year-old man, revealed a nodule in the right lobe of his prostate, which was considered to be malignant. After prostatectomy, pathologic examination showed adenocarcinoma of the prostate. Seven years prior to his operation, he had received 1,075 mg. of testosterone propionate during a period of twelve months.

In 27 men of the treated group prostatic hypertrophy had developed, and most of these patients had varying amounts of pus in the prostatic fluid. However, prostatectomy was necessary in only 5 of these 27, and microscopic examination of the tissues showed no evidence of carcinoma. Two patients, neither of whom presented any clinically detectable prostatic abnormality, died from unrelated causes, and postmortem examination failed to disclose any evidence of malignant change or nodule formation in the prostate gland. Five-year follow-up of the remainder of the treated patients who had been found to have palpable irregularities disclosed no evidence of carcinoma, with the exception of the 1 case previously mentioned. In the control group, palpable irregularities of the prostate were found in 13, and hypertrophy was noted in 34 patients.

Various pathologists (5, 6) have found that routine autopsy studies of the prostate of men over 50 years of age show small latent foci of carcinoma in 14 to 46 per cent. According to Dixon and Moore (7) careful studies of large series of unselected autopsy material place the incidence of carcinoma of the prostate in all men over the age of 40 at about 20 per cent.

SUMMARY

A study of the clinical effect of testosterone propionate on the prostate gland is presented. One hundred men over 45 years of age, previously treated for periods up to five years with amounts of testosterone propionate not exceeding 5,200 mg., were examined and the incidence of prostatic abnormalities compared with that in 100 controls. In 32 patients of the treated group, prostatic abnormalities had developed, but similar ab-

normalities had occurred in 39 of the control group. Twenty-seven treated patients showed prostatic hypertrophy, but 34 control subjects also had an enlarged prostate. Palpable prostatic irregularities occurred in 11 treated patients versus 13 control subjects. Carcinoma of the prostate was found only once—in an 80-year-old patient of the treated group, seven years after treatment was terminated. In this study, there was no evidence that testosterone propionate in the dosage used had initiated carcinoma or activated latent carcinoma of the prostate gland.

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COUNTER-CURRENT DISTRIBUTION PROCEDURE FOR THE QUANTITATIVE-QUALITATIVE ANALYSIS OF SMALL AMOUNTS OF URINARY CORTICOSTEROIDS*

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INTRODUCTION

THE present paper is an extension of previous work on procedures for the qualitative-quantitative analysis of small samples of urinary steroids of adrenocortical and gonadal origin (1). It involves the use of counter-current distribution discussed by Craig (2). The amount of thiosemicarbazide reactive material present is estimated by means of the thiosemicarbazide reaction which Evans and Gillam have shown to be useful for the detection of alpha-beta unsaturated ketones (3).

ANALYTIC PROCEDURE

1. *Reagents and apparatus*: Thiosemicarbazide reagent (Eastman Kodak Company). A 0.1 molar thiosemicarbazide solution in 0.1 molar hydrochloric acid is prepared and filtered into a glass-stoppered bottle. It is stable at room temperature for several months.

Ethanol, absolute (U. S. Industrial Chemicals Company)

Chloroform, reagent grade.

Sodium hydroxide, 0.1 N solution.

Secondary butyl alcohol (Eastman Kodak Company, highest purity), redistilled in an all-glass apparatus.

Iso-octane (2,2,4-trimethylpentane, pure grade 99 mol per cent; Phillips Petroleum Company, Chemical Products Division, Bartlesville, Oklahoma).

Solvent system for counter-current distribution: 400 ml. of distilled water, 300 ml. of iso-octane and 200 ml. of secondary butyl alcohol are mixed by shaking in a separatory funnel. The approximately equal upper and lower layers are allowed to settle and then drawn off separately.

Counter-current distribution apparatus: 25-tube, stainless steel, fitted with glass windows (4) (H. O. Post, Scientific Instrument Company, Maspeth, N. Y.). The tubes of the lower drum were calibrated according to the manufacturers' directions.

Spectrophotometer: Beckman model DU, equipped with ultra-violet light and 1-cm. quartz cuvettes.

2. *Procedure:* A chloroform extract of a fresh 24-hour urine collection is prepared

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after adjusting the pH to approximately 6 (5). The urine is extracted four times with 1/7 volume of chloroform. The chloroform is separated by centrifugation and washed three times with 1/10 volume of 0.1 N sodium hydroxide and three times with 1/10 volume of water. The residue[1] of the washed chloroform extract is dissolved in the contents of the zero tube of the distribution apparatus, which is filled with equal volumes of pure upper and lower layer solvents. The distribution is performed according to the general procedure described by Craig (2). The apparatus is gently inverted for forty-five seconds at each transfer and checked for the separation of the two layers by direct observation after one minute.

At the completion of the distribution, which takes about one hour, the material in each tube is transferred respectively to one of a series of 60-ml. wide-mouthed bottles. A reagent blank consisting of an equal volume of upper plus lower layer solvent is similarly treated. The bottles are placed in suitably shielded desiccator jars containing anhydrous calcium chloride as desiccant. After the jars have been evacuated for a period of at least two hours by means of a high-capacity, oil-type, motor driven vacuum pump, they are allowed to stand over night. If the residues are not completely dry by this time, residual solvent is removed by an additional period of evacuation.

The dry residues are dissolved in a measured volume of ethanol (usually 5 ml.) and the optical density of each at 302 millimicrons is determined with the aid of the spectrophotometer (Value A). Following this, a 3-ml. aliquot of each sample is transferred to a glass-stoppered test tube of approximately 15-ml. capacity; 0.5 ml. of thiosemicarbazide reagent is added and the material is mixed by gentle shaking. After the resultant mixture has stood at room temperature for ninety minutes, its optical density at 302 millimicrons is again measured. The value obtained is multiplied by the factor, 1.17, to correct for the dilution occasioned by the addition of reagent (Value B). The increase in optical density at 302 millimicrons (Value C or OD_{302}) attributable to thiosemicarbazones now is found by subtracting Value A from Value B.

RESULTS AND DISCUSSION

1. *Determination of absorption maxima:* The absorption spectrum of an ethanolic solution containing 10 micrograms per milliliter of 17-hydroxy-11-dehydrocorticosterone was determined before and after reaction with thiosemicarbazide. Figure 1 shows the absorption curves of the α,β-unsaturated ketone itself and of its thiosemicarbazone. It may be seen that the maximum in the region of 235–238 millimicrons due to the α, β-unsaturated ketone (Curve A) is shifted to the 300-millimicron region with the formation of the thiosemicarbazone (Curve B). Solutions of 17-hydroxycorticosterone, corticosterone and 11-dehydrocorticosterone gave identical results.

Solutions of the fractions obtained by counter-current distribution of a series of urine extracts from normal and abnormal subjects were studied also. The thiosemicarbazones formed with the substances present in fractions 1 through approximately 17 (Curve A, Figure 2) gave extinction-

[1] With the 25-tube apparatus, it is necessary to use thiosemicarbazide reactive material equivalent to between 300 and 900 micrograms of crystalline 11-dehydro-17-hydroxycorticosterone, in order to obtain satisfactory readings.

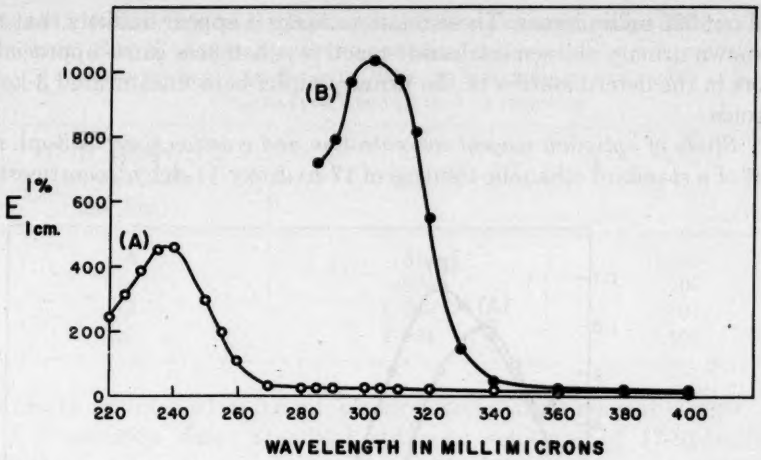

FIG. 1. Extinction-wavelength curves of crystalline 11-dehydro-17-hydroxycorticosterone in ethanolic solution before (Curve A) and after (Curve B) reaction with thiosemicarbazide. The abscissal scale gives the wavelength in millimicrons, and the ordinate scale indicates the light extinction expressed in terms of a 1 per cent solution in a 1-cm. cell.

wavelength curves very similar to Curve B of Figure 1. The curves obtained for fractions 17 through 24 were of the shape indicated by Curve B of Figure 2. Curve C of this figure was determined as the difference between Curves B and A. It may be seen that Curve C indicates the presence in certain counter-current distribution fractions of substances yielding thiosemicarbazones with negligible extinction at 300 and maximum extinction

TABLE 1. EFFECT OF VARYING (A) VOLUME OF REAGENT AND (B) REACTION TIME ON INTENSITY OF THIOSEMICARBAZIDE REACTION WITH 11-DEHYDRO-17-HYDROXYCORTICOSTERONE. THE AMOUNT OF STEROID USED IN EACH EXPERIMENT WAS 12.5 MICROGRAMS PER MILLILITER OF ETHANOLIC SOLUTION. THE RESULTS SHOWN ARE THE INCREASES IN OPTICAL DENSITY (O.D.) VALUES AT 302 MILLIMICRONS

Vol. of reagent (ml.)	Increase in optical density		
	Reaction time 60 min.	Reaction time 90 min.	Reaction time 120 min.
0.1	.820	.992	1.102
0.2	1.092	1.210	1.273
0.3	1.193	1.270	1.293
0.4	1.262	1.312	1.321
0.5	1.295	1.323	1.331

at about 320 millimicrons. These relations make it appear unlikely that the unknown urinary thiosemicarbazide-reactive substances cause appreciable errors in the determination of the urinary alpha-beta unsaturated 3-keto-steroids.

2. *Study of optimum reagent concentration and reaction time:* A 3-ml. aliquot of a standard ethanolic solution of 17-hydroxy-11-dehydrocorticoster-

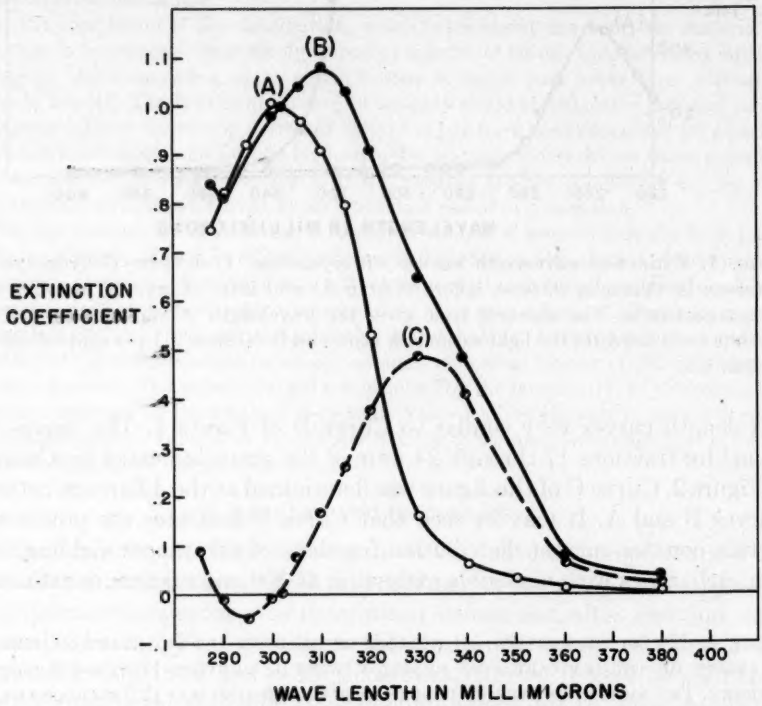

FIG. 2. Representative extinction-wavelength curves given by the thiosemicarbazones formed from extracts of normal and abnormal urine. The average of curves obtained for fractions 1–17 is given by Curve A and for fractions 18–24 by Curve B. Curve C is equal to Curve B minus Curve A.

one containing 12.5 micrograms per milliliter was transferred to each of five glass-stoppered test tubes. To each tube thiosemicarbazide reagent was added in amounts ranging from 0.1 to 0.5 ml. To provide suitable reagent blanks, five tubes were similarly prepared with 3-ml. lots of steroid-free ethanol. The optical densities of the reaction products were determined at 302 millimicrons at intervals of sixty, ninety, and one hundred and twenty minutes. As shown in Table 1, the reaction is essentially complete

TABLE 2. SAMPLE CALIBRATION DATA FOR THE CORTICOSTEROID-THIOSEMICARBAZIDE REACTION. THE K VALUES REPRESENT THE OPTICAL DENSITIES OF THE THIOSEMICARBAZONES FORMED, EXPRESSED IN TERMS OF 1 MICROGRAM OF STEROID IN A CELL HAVING A DEPTH OF 1 CENTIMETER

17-OH, 11-dehydro-corticosterone (μg./ml.)	Δ_{302}	K
5	.519	.104
10	1.026	.103
15	1.520	.101
20	2.054	.103

in ninety minutes when 0.5 ml. of thiosemicarbazide reagent is used.

3. *Calibration data:* Standard ethanolic solutions of 17-hydroxy-11-dehydrocorticosterone made to contain 5 to 20 micrograms per milliliter were treated according to the analytic procedure described. In Table 2 it

TABLE 3. MOLAR EXTINCTION COEFFICIENTS OF THE THIOSEMICARBAZONES AND PARTITION COEFFICIENTS OF VARIOUS CRYSTALLINE STEROIDS

Compound	Molar extinct. coeff. of thiosemicarb.	Partition coeff.		Site of max. conc. (tube number)
		by 1-tube method	by counter-current distrib.	
	[1]	[2]	[3]	[4]
11-Hydroxy-11-dehydrocorticosterone (E)	36,400	1.55	1.55	15
11-Desoxycorticosterone	39,900	17.80		23
Progesterone	38,300	6.40		21
11-Dehydrocorticosterone	38,000	1.91	2.00	16–17
Corticosterone (B)	35,500	3.73	4.20*	19–20
17-Hydroxycorticosterone (F)	36,700	1.40	1.35	14
Testosterone	33,500	11.00		22
Δ^4-Androstenedione	33,650	4.95		20
Dihydro E	945			
Allo-pregnanedione	430			
Estrone	430			
Dehydroisoandrosterone	430			
Allo-dihydro E	343			
Tetrahydro E	343			
Androsterone	86			
Pregnanediol 3α, 20α	8			

* Impure, according to distribution data (see Curve B, Figure 3).

may be seen that this thiosemicarbazone reaction follows Beer's law. An average K value of .103 with an error of ± 2 per cent was obtained.

4. *Specificity of reaction:* Various steroids were subjected to the thiosemicarbazide reaction. The results obtained, expressed in terms of molar extinction coefficients[2], are set forth in Table 3. It may be noted that steroids having an α,β-unsaturated ketone group in position 3 gave molar extinction coefficients at 302 millimicrons in the range of 33,500 to 40,000. Steroids having ketones which are not α,β-unsaturated gave negligible extinction coefficients at this wave length.[3]

5. *Partition coefficients of certain steroids:* Samples of crystalline pure steroids were dissolved in lower-layer solvent at a concentration of about 10 micrograms per milliliter. The exact concentration of steroid in each instance was determined spectrophotometrically by measuring the optical density of the lower-layer solvent at about 245 millimicrons.[4] A measured volume of the steroid solution was then shaken with an equal volume of upper-layer solvent. Following this equilibration the concentration of steroid in lower-layer solution was redetermined and decreases in concentration of steroid in this medium were assumed to be exactly equal to the concentration of steroid in upper-layer solution.

Partition coefficients calculated on the basis of this assumption are listed in column 2 of Table 3. Partition coefficients for a number of the substances studied were also determined by counter-current distribution by means of equation No. 7 of Williamson and Craig (6). The latter results, which are listed in column 3, are seen to be in close agreement with the former, except when the steroid was found to be impure by counter-current distribution. Both show that the four 11-oxycorticosteroids studied have partition coefficient values which range between about 1.4 and 4.0. The tube number in which the maximum concentration of substance of a given partition coefficient can be expected to appear is calculated from equation No. 5 of Williamson and Craig (6). As may be seen in column 4 of the table, these partition coefficient values mean that these substances appear in maximum concentration in tubes Nos. 14 to 19 of the counter-current distribution apparatus. Eleven-desoxycorticosterone with a partition coefficient of about 18 is well separated, appearing largely in tube No. 23. Androstenedione, progesterone and testosterone occupy intermediate positions with

[2] The increase in optical density at 302 millimicrons after reaction with thiosemicarbazide reagent, calculated for 1 mol per liter of steroid in ethanol in an absorption cell 1 cm. in length.

[3] Thiosemicarbazones of aldehydes and ketones without α,β-unsaturation give absorption maxima near 270 millimicrons (3) and show negligible absorption at 302 millimicrons.

[4] In lower-layer solution the absorption maxima of α,β-unsaturated 3-ketosteroids range between about 245 and 248 millimicrons.

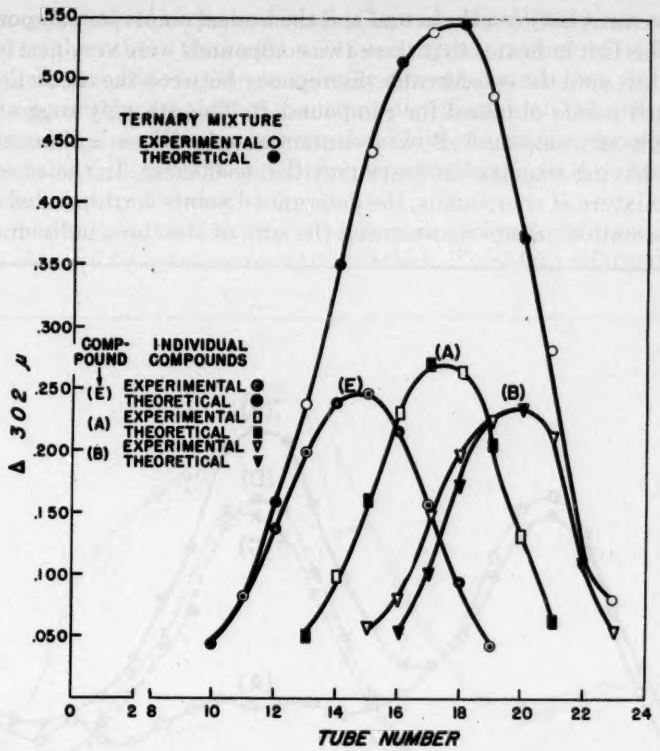

FIG. 3. Observations on the counter-current distribution of 74 micrograms of 11-dehydro-17-hydroxycorticosterone (E), 72 micrograms of 11-dehydrocorticosterone (A) and 66 micrograms of corticosterone (B), both singly and in ternary mixture. Note that every other point describing each curve, respectively, was determined experimentally. In the case of Curves E, A and B, the alternate theoretical points were calculated according to the directions of Williamson and Craig (6). In the case of the ternary curve, the theoretical curve is the sum of Curves E, A and B.

respect to partition coefficients and appear in maximum concentration in tubes Nos. 20 to 22.

6. *Counter-current distribution of crystalline corticosteroids:* The behavior in the counter-current distribution procedure of samples of 17-hydroxy-11-dehydrocorticosterone (compound E), 11-dehydrocorticosterone (compound A) and corticosterone (compound B), both singly and in ternary mixture, is illustrated in Figure 3. In each instance, both determined and theoretical[5] points were used in plotting the curves. Observe that there was

[5] The theoretical points were calculated according to the procedure of Williamson and Craig (6).

close agreement between the actual and theoretical points for compounds E and A. This fact indicates that these two compounds were very nearly pure. By contrast, note the considerable discrepancy between the theoretical and determined points obtained for compound B. This strongly suggests that the sample of compound B was contaminated with a α,β-unsaturated 3-ketone having a somewhat lower partition coefficient. In the case of the ternary mixture of compounds, the determined points corresponded closely to the theoretical points representing the sum of the three individual con-

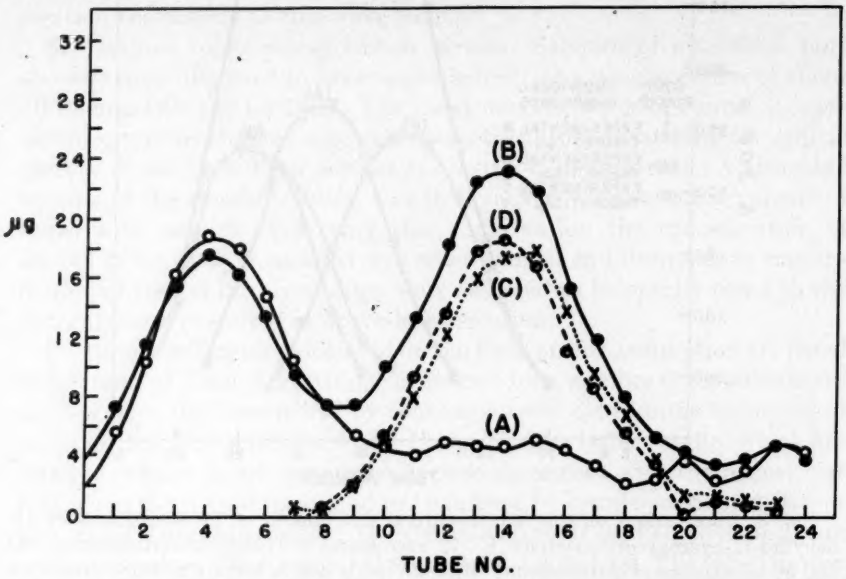

FIG. 4. Quantitative-qualitative study of recovery of cortisone (compound E) added to an aliquot of normal adult urine extract. Curve A = control, Curve B = control plus 113 mg. of cortisone, Curve C = 113 mg. of cortisone alone, and Curve D = Curve B minus Curve A.

stituents. This shows that the behavior of an individual corticosteroid in the counter-current distribution procedure is not altered appreciably by the presence of other corticosteroids in the solution being studied. The nature of the individual curves, of which such a ternary curve as that of Figure 3 represents the sum, can be deduced with reasonable accuracy by procedures described by Craig (7). It follows that the procedure is of potential value in the qualitative-quantitative analysis of steroid mixtures.

7. *Recovery of cortisone added to an extract of human urine:* For the purposes of this study, the extract of a 48-hour sample of adult urine was

divided into two equal aliquots. To one (Curve A, Fig. 4) there was no addition, but to the other (Curve B) a 113-microgram portion of crystalline compound E was added prior to counter-current distribution and assay. Curve C was obtained by counter-current distribution of 113 micrograms of compound E alone. It may be seen that Curve D, which represents the difference between Curves A and B, corresponds closely to the theoretical ideal represented by Curve C.

8. *Effect of varying the quantity of urinary extract material upon pattern of results obtained by counter-current distribution.* To obtain information on

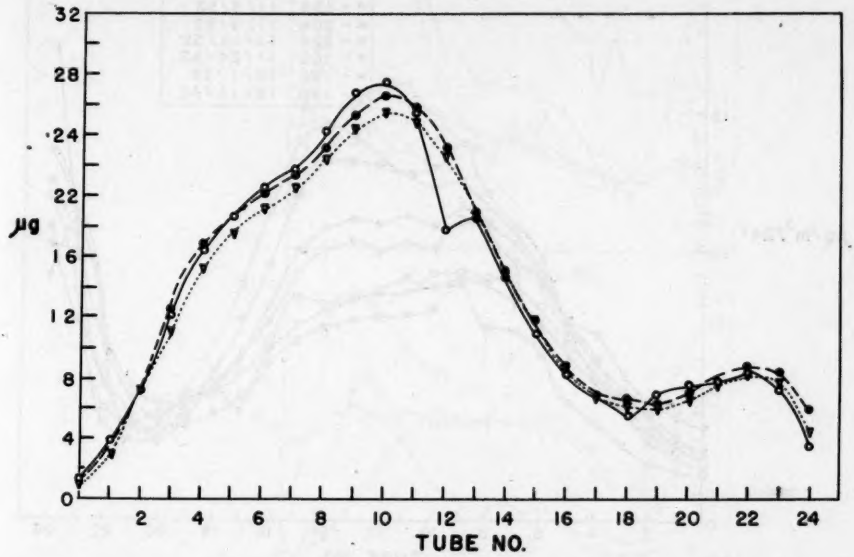

FIG. 5. Results obtained after counter-current distribution of 325-mg. (white circles), 650-mg. (black circles) and 975-mg. (triangles) aliquots of a single pool of urinary extract material. To permit direct comparison of the three distribution studies, the values actually obtained in the second experiment were divided by 2, and those for the third experiment by 3.

this subject, aliquots of a single urine extract pool containing, respectively, 325, 650 and 975 micrograms of thiosemicarbazide reactive material were subjected to counter-current distribution. To permit direct comparison of the three experiments, the results obtained in the case of the 650-microgram experiment were divided by 2, and those of the 975-microgram experiment by 3. Figure 5 shows that the curves representing these observations are nearly identical. It follows that studies carried out with quanti-

ties of material falling within this range should yield reasonably representative data.

9. *Observations on normal and abnormal individuals.* In Figure 6 are presented the results of a series of counter-current distribution studies carried out on 7 extracts of the urine of a normal young woman, collected at approximately weekly intervals. These data indicate that the urinary output

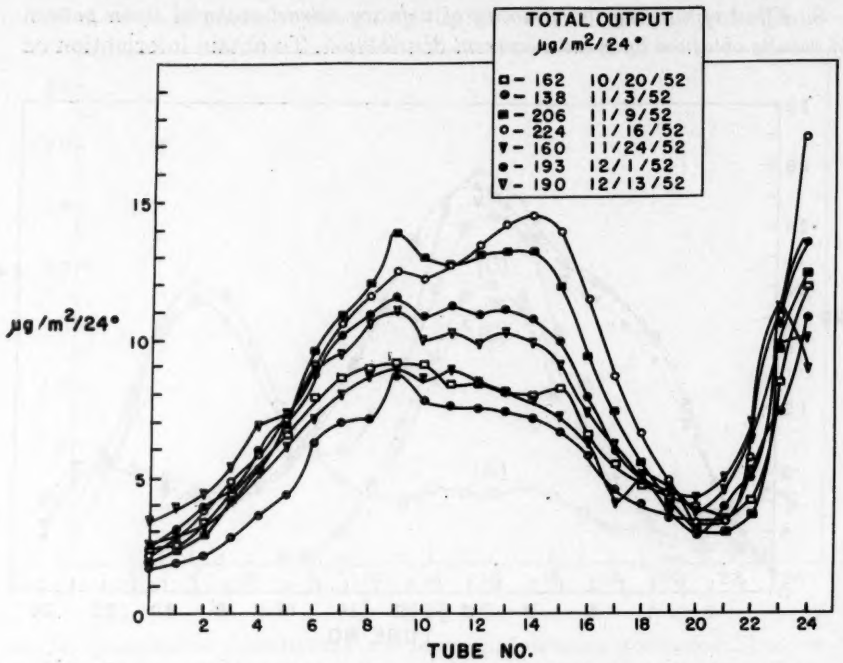

FIG. 6. Seven observations on the urinary output of thiosemicarbazide-reactive sub stances by a healthy young woman over an interval of eight weeks. The insert give values for total amounts of material excreted on the days represented by each curve. Menstruation occurred from November 3, 1952 to November 9, 1952 and from December 7, 1952 to December 13, 1952. The results are expressed as microgram equivalents of compound E per square meter per twenty-four hours.

of corticosteroid-like material by this individual did not undergo major quantitative or qualitative variations under conditions of health and a steady environment. No systematic relationship between the total output or the output of any group of fractions and the occurrence of menstruation could be discerned.

Figure 7 summarizes 15 observations on several healthy individuals. It is seen that persons of both sexes had approximately similar excretion

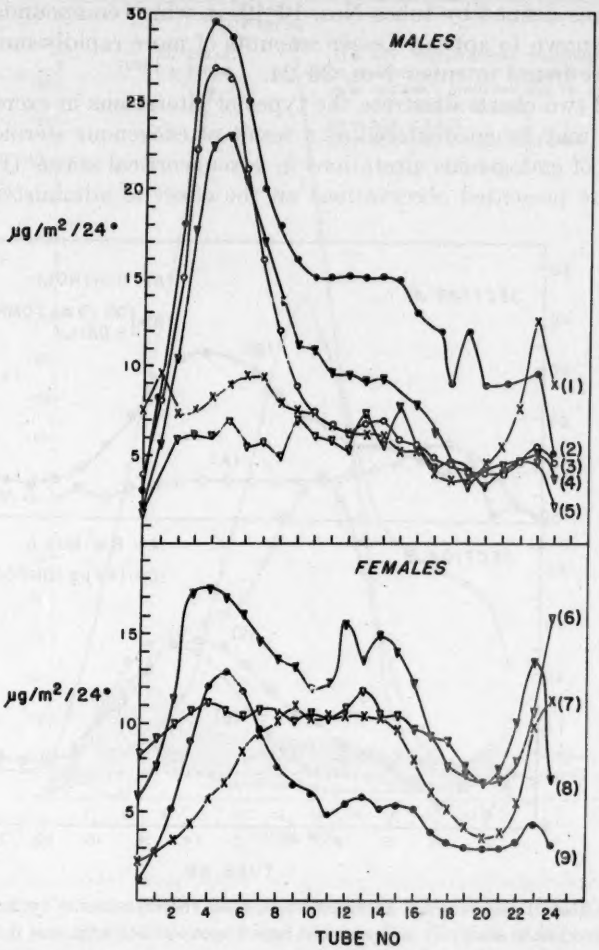

FIG. 7. Counter-current distribution of thiosemicarbazide-reactive substances excreted by 5 normal males and 3 normal females. The surface areas of the subjects of curves 1, 2, 3, 4 and 5 were 1.6, 0.4, 1.8, 1.9, 1.6 sq. M., respectively, and of the subjects of curves 6, 7 and 9, 1.6 sq. M. Curves 7 and 8 represent two measurements on the same person, the latter curve being obtained at a time when she was taking a low-sodium diet for study purposes. The results are expressed as microgram equivalents of compound E per square meter per twenty-four hours.

curves. Five had a definite excretion peak of moderate size in the region of tube No. 4. None of the crystalline compounds thus far studied has appeared in this region. Appreciable amounts of material also were present

in the regions defined by tubes Nos. 10–19, in which compounds A, B, E and F are known to appear. Lesser amounts of more rapidly moving substances were found in tubes Nos. 20–24.

The final two charts illustrate the types of alterations in excretion pattern which may be encountered as a result of exogenous steroid therapy (Fig. 8) or of endogenous alterations in adrenocortical status (Fig. 9). In Figure 8 are presented observations on the effect of administering com-

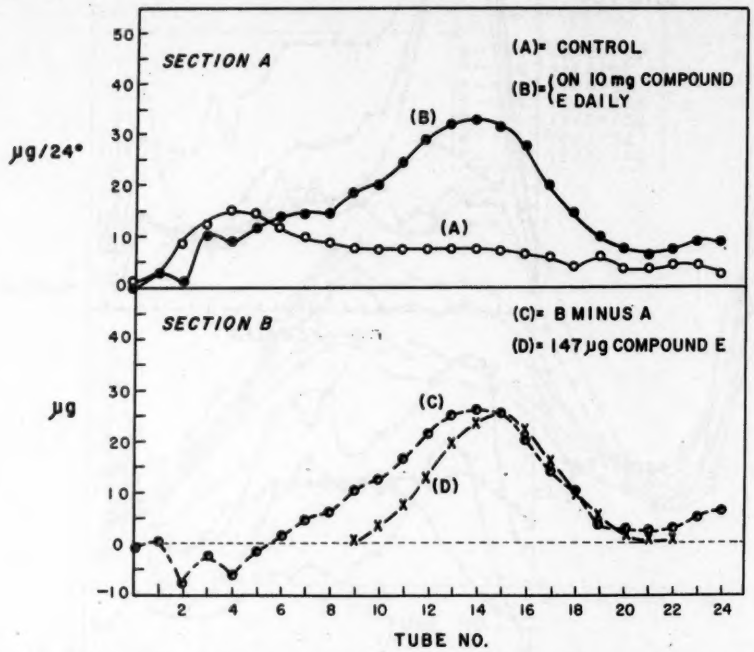

FIG. 8. Effect of compound E administration upon the excretion of corticosteroid-like substances (ordinate scale) by a 7-year-old boy whose surface area was 0.4 sq. M. (see text for descriptive comments).

pound E upon the urinary output of corticosteroid-like substances by a 7-year-old boy who was short and thin, but otherwise free from disease. Curve A indicates his output during a control period; it falls within the limits of normal previously described in Figures 6 and 7. Curve B gives his output during a period when he was taking compound E in daily doses of 10 mg. by mouth. Curve C, which gives the difference between B and A, may be compared to Curve D, which represents 147 micrograms of crystalline compound E. Analysis of Curve C would suggest that it is made up chiefly of substances having a distribution similar to that of compound E,

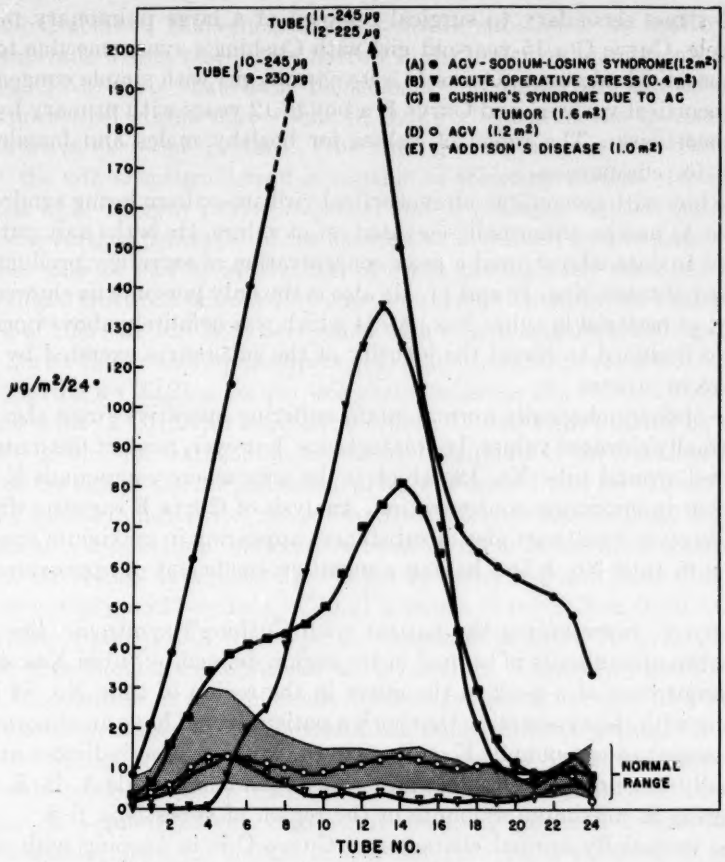
TUBE { 11-245 µg 12-225 µg
TUBE { 10-245 µg 9-230 µg
(A) ● ACV-SODIUM-LOSING SYNDROME(1.2m²)
(B) ▼ ACUTE OPERATIVE STRESS (0.4 m²)
(C) ■ CUSHING'S SYNDROME DUE TO AC TUMOR (1.6m²)
(D) ○ ACV (1.2m²)
(E) ▽ ADDISON'S DISEASE (1.0m²)
µg/m²/24°
NORMAL RANGE
TUBE NO.

FIG. 9. Counter-current distribution of urinary thiosemicarbazide-reactive substances excreted by patients with various conditions, as indicated in the key and in the text. The shaded area gives the normal range indicated by the data of Figures 6 and 7. The results are expressed as microgram equivalents of compound E per square meter per twenty-four hours.

and to a small extent of material appearing in maximum concentration in the region of tube No. 10. The free, corticosteroid-like urinary extract material represented by Curve C amounts quantitatively to approximately 1 per cent of the daily oral dose of compound E given to this patient.

In Figure 9 are shown measurements obtained on 5 patients with gross disturbances in adrenocortical status. Curve A represents a boy of 15 years with the congenital adrenocortical virilism-sodium losing syndrome (8), Curve B an endocrinologically normal 10-month-old infant suffering from

severe stress secondary to surgical removal of a large pulmonary pneumatocele, Curve C a 15-year-old girl with Cushing's syndrome due to an adrenocortical adenoma, Curve D a 9-year-old girl with simple congenital adrenocortical virilism, and Curve E a boy of 12 years with primary hypoadrenocorticism. The range of values for healthy males and females is shown for comparison.

The boy with congenital adrenocortical virilism-sodium losing syndrome (Curve A) had an abnormally elevated set of values. He is the only patient studied to date who showed a peak concentration of excretory products in the area of tubes Nos. 10 and 11. He also is the only person who showed an output of material in tubes Nos. 20–24 which was definitely above normal. Studies designed to reveal the identity of the substances excreted by this boy are in process.

The endocrinologically normal infant suffering operative stress also had abnormally elevated values. In this instance, however, most of the material centered around tube No. 13, which is the area where compounds E and F appear in maximum concentration. Analysis of Curve B suggests that it is made up in small part also of substances appearing in maximum concentration in tube No. 8 and having a partition coefficient of approximately 0.5.

Curve C, representing the patient with Cushing's syndrome, lies well above the upper limits of normal in the region defined by tubes Nos. 4–20. The occurrence of a peak in the curve in the region of tube No. 14 is in keeping with the expectation that such a patient would have an abnormally high output of compounds E or F, or both. Curve C also indicates an abnormally large output of substances other than compounds A, B, E or F appearing in maximum amounts in the region of tubes Nos. 6–8.

The essentially normal character of Curve C is in keeping with other data which suggest that children with simple congenital adrenocortical virilism do not produce abnormally large amounts of cortisone-like corticosteroids (9).

The values obtained for the hypoadrenocortical patient (Curve D) were subnormal in the region defined by tubes Nos. 9–21. This finding, together with the previously described data, suggests that this is the area wherein changes in adrenocortical activity are most strikingly revealed. The lack of a consistent relationship between adrenocortical status and output values observed for tubes Nos. 0–4 indicates that the materials usually appearing in this area may not be of adrenocortical origin. The same would seem to be true for tubes Nos. 21–24.

DISCUSSION

The data presented indicate not only that the procedure described yields reliably reproducible results, but also that the output of neutral, chloro-

form-extractable, thiosemicarbazide-reactive substances by healthy individuals falls within reasonably narrow limits under ordinary conditions of living. It is clear that both physiologic and pathologic alterations in adrenocortical status may result in major changes in the quantitative-qualitative excretion pattern. This finding is in keeping with the thesis that the adrenocortical gland is capable of secreting various steroid hormones with different physicochemical and physiologic characteristics.

Some further definition of the substances measured by the present procedure may be had by application of other steroid assay reactions to the individual fractions obtained by counter-current distribution. Preliminary work of this sort has been performed with blue tetrazolium (10) and with the Porter-Silber assay procedures (11). Blue tetrazolium reacts with steroids having a C20,21 α-ketolic side chain, whereas the Porter-Silber reaction is with 17-OH,20,21 α-ketolic steroids. The results obtained by application of the blue tetrazolium procedure to aliquots of the fractions represented by two of the normal male curves of Figure 7 and by Curves A and C of Figure 9 indicate that there is approximately as much C20,21 α-ketolic as there is Δ^4,3-ketonic material in most of these fractions. On the other hand, the results obtained with the Porter-Silber reaction show that there are essentially no 17-hydroxy C20,21 α-ketols in tubes Nos. 0–10, but appreciable quantities of this type of compound in tubes Nos. 11–24. The same phenomenon was observed when fractions obtained by counter-current distribution of enzymatically hydrolyzed urine extracts of a normal individual were assayed by means of the Porter-Silber reaction.

The findings presented here show that the present procedure can be used not only to detect major qualitative differences in urinary corticosteroid output in a quantitatively dependable manner, but also to determine in approximate terms the nature of the materials being excreted. It should therefore aid in the classification of individuals in terms of adrenocortical status and in the selection of urinary extract fractions for the purpose of detailed isolation and identification.

SUMMARY

A relatively simple procedure is described, applicable to 24-hour samples of urine, for the extraction, fractionation and spectrochemical assay of corticosteroid-like substances.

Clinical studies indicate that the quantitative-qualitative excretion pattern per square meter per day of healthy persons is steady within fairly narrow limits. Gross qualitative as well as quantitative changes in the output of urinary corticosteroid-like substances have been observed in a small series of preliminary observations on patients suffering either severe stress or definite adrenocortical disease. It would therefore appear that this procedure should be of practical value in clinical surveys of possible relation-

ships between the quality as well as the quantity of corticosteroid-like material appearing in urine and the adrenocortical status of the individual.

Acknowledgment

The authors are indebted to Drs. Edward C. Kendall, Konrad Dobriner, John K. Wolfe and to Dr. Erwin Schwenk of Schering Corporation, Dr. William J. Haines of the Upjohn Company and Dr. H. F. Colfer of Merck and Company for the supplies of steroid used in these studies. They wish also to acknowledge the technical assistance given by Mrs. Adrienne Campbell and the helpful advice given by Drs. Lewis Engel, Wilson R. Slaunwhite, Jr., Walter Eberlein and Miss Priscilla Carter of the Massachusetts General Hospital.

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17,21-DIHYDROXY-20-KETOSTEROIDS IN PLASMA DURING AND AFTER PREGNANCY

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THE beneficial effects of pregnancy on rheumatoid arthritis have been established (1). The prompt and dramatic improvement of patients with rheumatoid arthritis following the administration of cortisone, suggested to Hench (2) that the ameliorating effects observed during pregnancy may be due to the increased secretion of adrenocortical hormones of the glucocorticoid type. However, the underlying mechanism for the beneficial effect of pregnancy upon rheumatoid arthritis is still obscure (3–4). Recently Meyer and coworkers (5), studying the formation of granulation tissue induced by subcutaneous implanted cotton pellets in rats, found that during pregnancy there was a progressive reduction in the amount of granulation tissue formed, the least amount being formed at the time of parturition. The inhibition of granuloma formation noted by these authors is quantitatively similar to that occurring when cortisone or hydrocortisone is administered (6).

Gemzell (7) determined the blood levels of 17-hydroxycorticosteroids in pregnant women using the method of Nelson and Samuels (8), and concluded that the 17-hydroxysteroids were elevated during pregnancy, but the plasma levels dropped to normal within six days after delivery. This rapid fall in plasma concentration is not in keeping with the relatively prolonged beneficial effects of pregnancy in rheumatoid arthritis. Extensive studies by Hench (9) have shown that the typical relief pattern in pregnancy extends through 4 ± 3 weeks post partum. In view of the availability of a new and relatively simple chemical method for measuring plasma 17,21-dihydroxy-20-ketosteroids, it seemed of interest to measure the plasma levels of these substances during and after pregnancy.

MATERIALS AND METHODS

Of the 104 patients examined in this investigation, 71 were at different stages of pregnancy or at various time intervals post partum. For purposes of comparison, the 17,21-dihydroxy-20-ketosteroid levels in the plasma of

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TABLE 1. PLASMA LEVELS OF 17,21-DIHYDROXY-20-KETOSTEROIDS IN NORMAL PATIENTS

Patient	Sex	Age	Compound F in plasma (γ/100 ml.)
1	F	14	14
2	F	19	4
3	F	25	25
4	F	26	7
5	F	28	7
6	F	28	19
7	F	29	10
8	F	29	16
9	F	31	17
10	F	34	12
11	F	37	7
12	F	37	6
13	F	38	15
14	F	44	9
15	F	49	17
16	F	28	16
17	M	19	6
18	M	22	15
19	M	32	7
20	M	33	25
21	M	34	14
22	M	41	7
23	M	42	11
24	M	43	10
25	M	50	14
			$\bar{x} = 12 \pm 1.1$

25 normal subjects, and 8 nonpregnant females suffering from various disorders, are also presented. The 17,21-dihydroxy-20-ketosteroids were determined according to the method of Silber and Porter (10) on freshly drawn samples of plasma taken for the most part between 9 and 11 a.m.

RESULTS

Table 1 summarizes the findings in normal females and males. The mean level in this group was 12 ± 1.1[1] micrograms per 100 ml. of plasma. Plasma levels in both sexes were essentially of the same order. Except in 3 in-

[1] Standard error $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

TABLE 2. 17,21-DIHYDROXY-20-KETOSTEROID LEVELS IN NONPREGNANT FEMALES SUFFERING FROM VARIOUS DISORDERS

Patient	Age	Compound F in plasma (γ/100 ml.)	Remarks
1	36	18	Menopausal syndrome
2	44	10	Hypertension
3	65	21	Hypertension
4	41	17	Diabetes
5	47	10	Breast tumor
6	53	14	Common cold
7	46	11	Menopausal syndrome
8	44	19	Chronic cystic mastitis
		$\bar{x}=15\pm1.5$	

stances in which plasma levels from 19 to 25 micrograms per 100 ml. were found, all values were below 18 micrograms per 100 ml. The findings in these normal subjects are in close agreement with those reported by Bliss (11), who employed the method of Nelson and Samuels (8). In addition, the plasma levels in a group of nonpregnant females suffering from various disorders were within the range observed in the normal control subjects (Table 2). Similar results have been reported by Perkoff *et al.* (12).

Table 3 shows the values in 35 pregnant patients. In all but 2 of these patients the 17,21-dihydroxy-20-ketosteroid plasma levels were significantly elevated. A mean value of 33 ± 1.4[1] micrograms per 100 ml. of plasma was found. Although sufficient data are not available to draw final conclusions, it would appear that the 17,21-dihydroxy-20-ketosteroid plasma levels are of the same order of magnitude in early and late stages of pregnancy.

Postpartum studies. There is a moderate, but definite, decrease in the plasma level of 17,21-dihydroxy-20-ketosteroid after delivery (Table 4). The mean value for this group of patients was 22 ± 1.3 micrograms per 100 ml. of plasma. In some cases the concentration fell to within the normal range a few days after delivery, but most of the patients still had slight to moderately elevated plasma levels six to nine weeks post partum.

COMMENT

Recently Wheeler *et al.* (13) reported on the increased urinary "corticoids" during pregnancy. The observations presented in this report are in agreement with those reported by Gemzell and show that the 17,21-dihydroxy-20-ketosteroid plasma levels are significantly elevated during

TABLE 3. 17,21-DIHYDROXY-20-KETOSTEROID LEVELS IN PREGNANCY

Patient	Age	Duration of pregnancy (mos.)	Compound F in plasma (γ/100 ml.)
1	15	2½	17
2	17	3	26
3	29	4	29
4	18	5	29
5	26	5	40
6	21	5	25
7	19	6	31
8	22	6	35
9	22	6	31
10	23	6	34
11	26	6	32
12	36	6	33
13	21	7	42
14	22	7	29
15	38	7	29
16	23	8	38
17	29	8	49
18	17	9	48
19	17	9	38
20	19	9	47
21	20	9	49
22	20	9	26
23	22	9	31
24	24	9	29
25	26	9	12
26	26	9	37
27	27	9	35
28	27	9	36
29	27	9	26
30	28	9	26
31	29	9	28
32	29	9	37
33	29	9	42
34	32	9	27
35	35	9	32

$\bar{x} = 33 \pm 1.4$

TABLE 4. 17,21-DIHYDROXY-20-KETOSTEROID LEVELS POST PARTUM

Patient	Age	Time after delivery	Compound F in plasma (γ/100 ml.)
1	19	4 hrs.	52
2	24	6 hrs.	32
3	30	10 hrs.	23
4	27	1 day	19
5	28	2 day	26
6	22	3 day	19
7	24	3 day	16
8	26	3 day	20
9	21	4 day	16
10	29	4 day	21
11	26	5 day	24
12	18	6 day	22
13	18	6 wks.	14
14	18	6 wks.	33
15	20	6 wks.	14
16	21	6 wks.	15
17	22	6 wks.	22
18	23	6 wks.	18
19	24	6 wks.	11
20	26	6 wks.	35
21	24	6 wks.	21
22	29	6 wks.	17
23	30	6 wks.	18
24	37	6 wks.	17
25	22	7 wks.	15
26	23	7 wks.	25
27	23	7 wks.	28
28	28	7 wks.	25
29	31	7 wks.	20
30	33	7 wks.	28
31	24	9 wks.	28
32	22	9 wks.	19
33	29	9 wks.	24
34	34	9 wks.	25
35	24	12 wks.	7
36	28	12 wks.	28
			$\bar{x} = 22 \pm 1.3$

pregnancy. After delivery, the plasma levels tend to fall, although in many patients the values were still somewhat elevated six to nine weeks post partum. These findings are in agreement with the hypothesis that the beneficial effects of pregnancy on rheumatoid arthritis are related to an increased blood level of hydrocortisone. The subsequent return of the signs and symptoms of rheumatoid arthritis post partum are also in keeping with the fall in steroid levels post partum. That pregnancy does not benefit all patients with rheumatoid arthritis and that there is considerable variation in the degree of relief obtained by patients with rheumatoid arthritis is undoubtedly due, in part, to the stage and activity of the disease at the time of pregnancy, but may also be related to the variation in the plasma 17,21-dihydroxy-20-ketosteroid concentration, as noted in the present study. The precise answer to the foregoing question can only be obtained by following the plasma levels of patients with rheumatoid arthritis during pregnancy and correlating such laboratory data with clinical findings.

SUMMARY

1. Plasma 17,21-dihydroxy-20-ketosteroid levels are significantly elevated during pregnancy and fall toward normal post partum.

2. The relationship of pregnancy and the plasma level of 17,21-dihydroxy-20-ketosteroid to the relief of rheumatoid arthritis is discussed.

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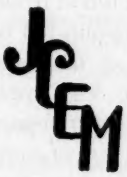

ANALYSIS OF HUMAN ADRENAL VEIN BLOOD AND ADRENAL GLANDS FOR STEROIDAL SUBSTANCES*

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IN 1950, Reich, Nelson and Zaffaroni (1) reported the presence of Kendall's compound F (17-hydroxycorticosterone) in the adrenal vein blood of dogs stimulated with ACTH. Two years later, Zaffaroni and Burton (2) described quantitative estimation of both compounds F and B in the adrenal vein blood of dogs. The concentration of compound F was always greater than that of compound B. A species difference in adrenocortical secretions was observed by Bush (3), who found the ratio of compound F to compound B to be from less than 0.05 in the rat to greater than 20 in the rhesus monkey.

Sweat *et al.* (4) examined the effluent blood from an adrenal gland of a patient with carcinoma of the breast and of a patient with Cushing's syndrome. The effluent adrenal blood from the former contained 290 μg. of compound F and 80 μg. of compound B per 100 ml., whereas the effluent from the latter contained 850 μg. of F and 236 μg. of B per 100 ml. The ratio of F to B in either case was approximately 3.6. More recently, Romanoff, Hudson and Pincus (5) reported the isolation of 4.5 mg. of crystalline compound F and 0.4 mg. of compound B from 500 ml. of effluent adrenal venous blood prior to adrenalectomy, in a patient with metastatic prostatic carcinoma. These values are respectively equivalent to 950 μg. of compound F and 80 μg. of compound B per 100 ml. The ratio of F to B in this case was approximately 11.9.

In view of these facts, it has seemed worthwhile to continue such studies. The results of an analysis of four samples of human adrenal effluent blood are reported here. In addition, analytical data concerning surgically excised human adrenal glandular tissue have been included.

EXPERIMENTAL

Female patients with metastatic carcinoma of the breast were used for this study. Prior to adrenalectomy, the adrenal vein was cannulated and the effluent blood was collected. The blood was immediately taken to the laboratory and diluted with 2 volumes

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of 50 per cent aqueous methanol. The steroids were then extracted with methylene chloride by a simultaneous dialysis and extraction technique previously described (6).

Sections of the adrenal glands excised from the same patients were set aside for histologic examination and the remaining tissue was homogenized in distilled water (20 per cent homogenate). Each adrenal homogenate was diluted with 2 volumes of 50 per cent aqueous methanol and also extracted by the foregoing method.

A preliminary fractionation of the methylene chloride extracts was carried out by paper chromatography, according to the scheme described by Zaffaroni (7). Each fraction was then chromatographed in the appropriate solvent system, utilizing the method of Romanoff *et al.* (8). All steroid-containing zones were located on the dried papergrams by techniques previously described (8). In addition, the NaOH fluorescence reaction described by Bush (9) for α,β-unsaturated 3-ketosteroids and the longwave ultraviolet (Raymaster) lamp for fluorescent areas were utilized. To detect the presence of phenols on the papergrams, the phenol reagent of Folin and Ciocalteu (10) as adapted in our laboratories (11) was employed.

The quantitative estimation of α,β-unsaturated 3-ketosteroids was carried out by determining in absolute methyl alcohol the ultraviolet absorption spectra of the isolated steroids and their concentration calculated on the basis of their densities at 240 mμ. The procedure described by Zaffaroni and Burton (2) was followed. When possible, determinations were made of the absorption spectra in sulfuric acid between 220 and 600 mμ (12).

The identification of compounds F and B was based on their chromatographic behavior, including that in mixed chromatograms with authentic crystalline samples of compounds F and B respectively, and on their spectrophotometric properties in absolute methanol and in concentrated sulfuric acid (12). In addition, these compounds gave the characteristic color reactions on papergrams, such as the blue tetrazolium (BTZ) reaction (13) for α-ketols, the orange color with dinitrophenylhydrazine (DNPH) for α,β-unsaturated ketones (1), and the NaOH fluorescence (14) for α,β-unsaturated 3-ketosteroids.

RESULTS

The adrenal vein blood of 3 female patients with carcinoma of the breast was analyzed. In Table 1 are presented data on the quantities of compounds F and B found in the effluent of the right adrenal. The adrenals of Patient No. 1 and Patient No. 3 appeared normal on histologic examina-

TABLE 1. QUANTITIES OF COMPOUND F AND COMPOUND B IN HUMAN RIGHT ADRENAL VEIN BLOOD

Patient No.	Sample No.	Blood vol. (ml.)	Compound F (μg.)		Compound B (μg.)		Ratio F/B
			Total	Per 100 ml.	Total	Per 100 ml.	
1	a	50	503	1006	121	242	4.13
	b	126	860	682	294	233	2.93
2	c	112	284	253	—	—	—
3	d	15	53	353	5.0	33.3	10.60

TABLE 2. TRACE SUBSTANCES DETECTED IN HUMAN ADRENAL VEIN BLOOD

Compounds*	Characteristics
Unknown No. 1	Remained at line of application when chromatographed for a 72-hour period in the system benzene saturated with formamide. Positive reaction for a phenol. Sulfuric acid chromogen had a shoulder at 250–260 mμ and maxima at 325 and 400 mμ.
Unknown No. 2	A maximum in absolute methanol at 260 mμ and a maximum in sulfuric acid at 260–265 mμ.
Compound F	
Unknown No. 3	Light blue fluorescent area.
Unknown No. 4	Yellow color with DNPH, characteristic of saturated ketones. A maximum in absolute methanol at 268 mμ and maxima in sulfuric acid at 264 and 304 mμ.
Unknown No. 5	Positive BTZ reaction for α-ketols. Orange color with DNPH characteristic of α, β-unsaturated ketones. A maximum in absolute methanol at 236 mμ.
Compound B	
Unknown No. 6	Fluorescent area with maximum in absolute methanol at 225 mμ and maxima in sulfuric acid at 266, 304, and 404 mμ.
Unknown No. 7	α, β-Unsaturated 3-ketosteroid with maximum in methanol at 239 mμ and a maximum in sulfuric acid at 280 mμ.
Unknown No. 8	Zimmermann reaction on papergram, typical of a 17-ketosteroid. Maxima in sulfuric acid at 274 and 320 mμ.
Unknown No. 9	Zimmermann reaction on papergram, typical of a 17-ketosteroid. Maxima in sulfuric acid at 272, 324, 390?, and 400? mμ.
Unknown No. 10	Green fluorescent area under longwave ultraviolet lamp.
Unknown No. 11	Green fluorescent area under longwave ultraviolet lamp.
Unknown No. 12	Yellow fluorescent area under longwave ultraviolet lamp.

* Listed in order of decreasing polarity.

tion. In Patient No. 2, metastatic tumor had replaced almost all of the medulla and much of the cortex of the left adrenal, whereas the right adrenal was only partially involved. The diagnosis was carcinoma in the adrenal glands following carcinoma of the right breast.

All patients received 150 to 200 mg. of cortisone acetate by mouth in the twenty-four hours preceding adrenalectomy. Two samples of right adrenal vein blood were collected from Patient No. 1. The first, consisting of 50 ml., was collected in forty-five minutes. The second sample, consisting of 126 ml., was collected over a period of one hour during the administration of 75 units of ACTH intravenously. From Patients Nos. 2 and 3, 112 and 15 ml., respectively, of right adrenal vein blood were collected.

It may be seen from Table 1 that the second sample of adrenal vein blood from Patient No. 1 contained less compound F per unit volume during ACTH administration than did the first sample obtained immediately after cannulation of the adrenal vein. The amount of compound B in both

TABLE 3. TRACE SUBSTANCES DETECTED IN HUMAN ADRENAL GLANDS

Compounds*	Characteristics
Unknown No. 1	Remained at line of application when chromatographed for a 72-hour period in the system benzene saturated with formamide. Positive reaction for a phenol. Maximum in absolute methanol at 250 mμ and maxima in sulfuric acid at 235 and 310 mμ.
Unknown No. 2	Negative reaction to BTZ; but a maximum in absolute methanol at 260 mμ and in sulfuric acid at 262 mμ.
Compound F	
Unknown No. 3	Yellow fluorescent area.
Unknown No. 4	A maximum in absolute methanol at 240 mμ. The quantity found was approximately 44 μg. per 10 Gm. or 4.4 μg. per Gm. of adrenal tissue. Maxima in sulfuric acid at 286, 350, and 410 mμ. Similar to compound A in running rate and sulfuric-acid chromogen.
Unknown No. 5	Yellow fluorescent area.
Unknown No. 6	A maximum in absolute methanol at 240 mμ. Approximately 325 μg. found in a gland weighing 10 Gm. Maxima in sulfuric acid at 290 and 430 mμ same as those for Δ^4-pregnene-17α, 20β, 21-triol-3-one.
Unknown Nos. 7, 8 & 9	Yellow fluorescent spots.
Unknown No. 10	Zimmermann reaction on papergram, typical of a 17-ketosteroid. Maxima in sulfuric acid at 270 and 320 mμ.
Unknown Nos. 11, 12 & 13	Yellow fluorescent spots.

* Listed in order of decreasing polarity.

samples, however, was practically identical. The sample of blood from the right adrenal of Patient No. 2 contained only 253 μg. of compound F per 100 ml. and no compound B could be detected. This gland was partially replaced by tumor. The amount of compound F in the right adrenal vein blood of Patient No. 3 was somewhat higher than in Patient No. 2 but was still a great deal lower than in Patient No. 1. A small amount of compound B was detected in the latter gland.

In Table 2 are listed a number of trace substances detected in the samples of adrenal vein blood analyzed. They are listed in the order of decreasing polarity. Unknown No. 1 was a phenol which remained at the base line of application when chromatographed for a 72-hour period in the system benzene saturated with formamide. Several substances which fluoresced under the longwave ultraviolet lamp were also detected. Unknowns Nos. 8 and 9 gave papergram Zimmermann reactions typical of 17-ketosteroids.

Six human adrenals were analyzed by the aforementioned techniques. All of them gave a spot comparable to compound F with characteristic color reactions for an α-ketol and an α,β-unsaturated 3-ketosteroid. The absorption spectra of the eluted spots in absolute methanol showed maxima

at 241–242 mμ. The sulfuric-acid spectra showed maxima at 240, 280, 390 and 475 mμ, identical with those for compound F. The amount of compound F found varied from 2.3 to 5.5 μg. per Gm. of adrenal tissue.

In Table 3 are listed a number of other trace substances which were detected in addition to compound F. Unknown No. 1 was a phenol which remained at the base line of application when chromatographed for a 72-hour period in the system benzene saturated with formamide. Again, several yellow fluorescent areas were detected under the longwave ultraviolet lamp. Approximately 4.4 μg. of unknown No. 4 per Gm. of adrenal tissue was detected in the right adrenal of Patient No. 1. It had maxima in sulfuric acid at 280, 350 and 410 mμ, and was similar to compound A (11-dehydrocorticosterone) in running rate and sulfuric-acid spectrum. Unknown No. 6 was an α,β-unsaturated 3-ketosteroid with a maximum in absolute methanol at 240 mμ; the concentration was approximately 325 μg. per 10 Gm., or 32.5 μg. per Gm. in the right adrenal of Patient No. 1. The maxima in sulfuric acid at 290 and 430 mμ were identical with those for Δ^4-pregnene-17α,20β,21-triol-3-one. However, it must be mentioned that before extraction this gland was perfused *in vitro* with Δ^4-pregnene-17α-ol-3,20-dione; therefore the Δ^4-pregnene-17α,20β,21-triol-3-one may very well have been a metabolite of this steroid. Unknown No. 10 gave a Zimmermann reaction typical for a 17-ketosteroid and had maxima in sulfuric acid at 270 and 320 mμ. This substance was detected in the right adrenal gland of Patient No. 2; this gland had been partially replaced by tumor.

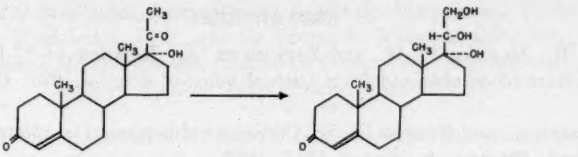
DISCUSSION

It was found that the second sample of adrenal vein blood from Patient No. 1, taken during ACTH administration, contained less compound F per unit volume than did the first sample obtained immediately after cannulation of the adrenal vein. A rational explanation of this finding cannot be offered. The adrenal gland of Patient No. 2 was partially involved with tumor and the blood from this gland had the lowest concentration of compound F per unit volume and no compound B.

Sweat *et al.* (4) reported finding 290 μg. of compound F per 100 ml. of effluent adrenal blood in a patient with carcinoma of the breast. This is comparable to the lowest value obtained in this study. He also reported the presence of 850 μg. of F per 100 ml. in the adrenal vein blood of a patient with Cushing's syndrome, and this is comparable to the amount which Romanoff, Hudson and Pincus (5) isolated from the adrenal vein blood of a patient with metastatic prostatic carcinoma. It is also slightly lower than the level reported here in the first sample from Patient No. 1. These data indicate the wide scope of variation. No real conclusion can be drawn as the to quantitative output of the human adrenal gland until a

sufficient number of samples of adrenal vein blood, under various conditions, have been analyzed.

Information on the presence of steroidal substances within the human adrenal gland has been lacking. An attempt to add to our knowledge was made here by individual analysis of 6 human adrenal glands. The presence of compound F was established in each one of these glands; the levels varied from 2.3 to 5.5 μg. per Gm. of tissue (wet weight). A substance with a running rate and sulfuric-acid spectrum similar to that of compound A



Δ^4-PREGNENE-17α-OL-3,20-DIONE Δ^4-PREGNENE-17α,20β,21-TRIOL-3-ONE

FIGURE 1

(11-dehydrocorticosterone) was also detected in one gland. A 17-ketosteroid was detected in a gland which was partially involved with tumor, but not in the others.

From one gland (Patient No. 1), we were able to isolate 325 μg. of a substance that had a sulfuric-acid spectrum identical with that of Δ^4-pregnene-17α,20β,21-triol-3-one. Before extraction, this gland was perfused *in vitro* with Δ^4-pregnene-17α-ol-3,20-dione; thus the isolated substance may have been an adrenal metabolite of the latter steroid. As shown in Figure 1, this would necessitate hydroxylation at position C-21. In addition, the C-20 ketone would have to be reduced to a C-20 hydroxyl. The evidence in favor of C-21 hydroxylation by the *in vitro* perfusion of the isolated bovine adrenal has been reviewed (15).

In addition, green and yellow fluorescent areas were detected under the longwave ultraviolet lamp in the adrenal vein blood and in all the adrenal glands examined. The yellow fluorescent substances appeared with amazing regularity and with exactly the same Rf values in each gland examined, whether it was involved with tumor or not. The area generally occupied by compound B on the papergram was always replaced by one of these yellow fluorescent spots in all the adrenal glands examined. This is interesting, since Eades *et al.* (16) have reported the isolation of blue and yellow-green fluorescing substances under longwave ultraviolet light from normal male urine. They have postulated that these substances are amino acid-steroid conjugates and have identified glycine in one of the amino acid conjugates. As we have isolated similar substances from human urine, we are at present investigating this possibility.

SUMMARY

Quantitative data on the rate of secretion of compound F (17-hydroxycorticosterone) and compound B (corticosterone) by the human adrenal are presented, based on analyses of adrenal vein blood.

Compound F was found to be the main steroid in the 6 human adrenal glands analyzed, the concentration varying from 2.3 to 5.5 micrograms per gram of adrenal tissue (wet weight). The presence of several other unidentified trace substances was also noted.

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THE INFLUENCE OF SINGLE DOSES OF PROPYLTHIOURACIL ON THE KINETICS OF IODINE ACCUMULATION BY THE THYROID GLANDS OF NORMAL AND HYPERTHYROID SUBJECTS*

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DURING the past decade, broad clinical experience has amply confirmed the efficacy of drugs of the thiouracil series in the amelioration of thyrotoxicosis (1-6). Studies in animals and man have revealed that agents of this chemical configuration do not inhibit the ability of the gland to concentrate inorganic iodide, but do interfere with the ability of the gland to synthesize hormone from its inorganic substrate (7–11).

Throughout this period, it has not been possible directly to quantify the effect of these agents on thyroidal function, since the protein-binding rate, which they affect, could not be measured. A new method has made possible measurement of the rate of organification of thyroidal iodide (12). This method has been employed in studying quantitatively the inhibition of protein-binding of thyroidal iodide produced in 18 patients by varying doses of propylthiouracil.

MATERIAL AND METHODS

Euthyroid and hyperthyroid individuals, and 2 patients with nontoxic goiter, comprised the 18 subjects. By a method fully described elsewhere (12), measurements of thyroidal organic-binding rate were made, prior to and following the administration of a single oral dose of propylthiouracil. Seven patients received 100 mg., 7 received 50 mg., and 4 received 25 mg. The second test was performed within ten days of the first test, and was begun from one to one and a half hours after the administration of the drug. Data obtained during the second test were corrected for the residual radioactivity in the plasma and thyroid gland, determined prior to the administration of the second dose of I^{131}.

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Early observations revealed that the effect of the antithyroid agent was maximal approximately three hours after its oral administration. Consequently, data obtained beyond this period were not used in subsequent calculations. In euthyroid individuals, therefore, the second tests were necessarily more brief than the control test. In hyperthyroid patients, the rapidity of both the thyroidal uptake and the disappearance of radioiodine from the plasma frequently made the control test very short. The diminution in thyroidal function produced by propylthiouracil frequently made possible a prolongation of the second test, as compared to the first.

RESULTS

In addition to the thyroidal iodide protein-binding rate, values for thyroidal iodide space, plasma radioiodide disappearance rate, thyroidal iodide

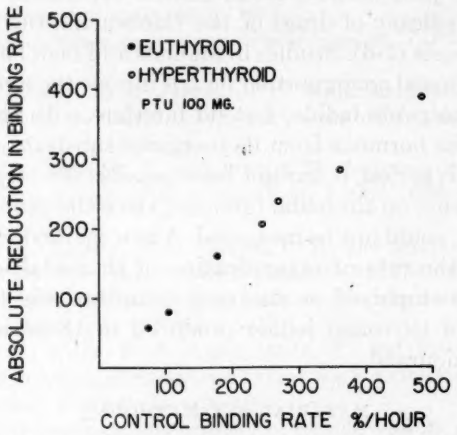

FIG. 1. The relationship between the reduction in protein-binding rate induced by 100 mg. of propylthiouracil and the control value of this function. No difference between the response of euthyroid and hyperthyroid patients is noted.

transfer rate, and the ultimate thyroidal uptake of I^{131} were measured before and after the administration of propylthiouracil (Table 1).

Protein-binding Rate (K): In attempting to assess the effect of propylthiouracil on thyroidal protein-binding of iodide, calculations were made of both the inhibited binding rate (residual binding rate) and the difference between the control and inhibited binding rates (absolute reduction in binding rate). At each dosage of propylthiouracil, these values were found to bear a highly significant statistical correlation with the magnitude of the control binding rate (Fig. 1). Since the magnitude of the inhibitory effect appeared to be proportional to the initial level of protein-binding activity,

TABLE 1. THE EFFECT OF SINGLE DOSES OF PROPYLTHIOURACIL ON THE KINETICS OF IODINE ACCUMULATION BY NORMAL AND HYPERFUNCTIONING THYROID GLANDS

Patient	Diagnosis	TIS* (liters)		K (% TIS/hour)				T_T (ml./min.)		I_U (% dose I^{131})		IDS (liters)		k (proportion/hour)	
		Control	% change	Control	Residual	Absolute reduction	% change	Control	% change	Control	% change	Control	% change	Control	% change
Propylthiouracil, 100 mg.															
J.C.	Graves' disease	3.5	+11.3	270.0	31.5	238.5	−88.3	156.4	−86.9	92.2	−69.2	19.4	−16.0	.525	− 49.5
D.H.	Graves' disease	10.9	− 5.8	242.3	37.0	205.3	−84.7	438.7	−85.6	92.3	−60.8	24.8	+ 0.9	1.150	− 63.5
M.S.	Graves' disease	7.4	+ 7.2	485.0	98.0	387.0	−79.8	601.6	−78.5	91.1	−33.1	20.6	+ 9.2	1.920	− 70.6
E.T.	Graves' disease	18.2	−16.0	363.0	82.2	280.8	−77.4	1100.0	−80.8	99.9	−21.7	34.0	− 4.7	1.940	− 74.2
B.Am.	Normal	1.4	− 9.8	103.6	24.0	79.6	−76.8	23.5	−79.2	27.1	−59.0	20.0	0	.260	− 48.8
L.H.	Normal	0.8	+11.1	178.0	18.2	159.8	−89.8	23.8	−88.7	43.2	−82.4	18.2	+ 2.2	.182	− 36.8
L.E.	Nontoxic goiter	1.1	0	75.0	18.4	56.6	−75.4	13.7	−75.4	24.8	−77.8	21.6	+10.6	.154	− 0.6
	Mean		*− 0.3*		*44.2*	*201.1*	*−81.7*		*−82.1*		*−57.7*		*+ 0.3*		*− 49.1*
	Std. Dev.		*10.7*		*32.4*	*115.2*	*5.8*		*5.0*		*22.6*		*8.9*		*25.2*
Propylthiouracil, 50 mg.															
M.N.	Graves' disease	5.5	+ 2.6	560.0	376.0	184.0	−67.1	513.2	−66.3	92.1	−26.9	23.8	0	1.405	− 53.9
M.R.	Graves disease	6.7	+ 7.8	221.0	81.0	140.0	−36.6	248.1	−31.7	84.8	−14.5	28.4	+13.7	.618	− 29.9
E.B.	Normal	1.0	+ 6.8	124.0	68.0	56.0	−54.9	21.3	−51.7	21.6	−68.5	14.7	+ 7.2	.402	+ 49.5
M.Co.	Normal	1.6	− 6.6	87.0	64.0	23.0	−73.6	23.0	−75.2	30.5	−73.3	17.2	+ 5.8	.262	− 11.5
M.G.	Normal	1.4	0	116.4	84.4	32.0	−72.4	27.1	−72.4	38.8	−31.2	19.2	−13.7	.222	− 57.2
L.S.	Normal	0.9	+11.9	75.0	35.0	40.0	−46.6	11.0	−36.4	31.8	−72.0	16.7	−14.4	.125	+161.8
N.T.	Nontoxic goiter	1.0	+ 4.2	50.7	19.7	31.0	−38.8	8.4	−35.7	26.0	−66.9	12.5	+ 0.8	.156	+ 90.3
	Mean		*+ 5.8*		*104.0*	*72.3*	*−55.7*		*−52.8*		*−50.5*		*− 0.1*		*+ 21.3*
	Std. Dev.		*5.9*		*122.2*	*63.4*	*15.6*		*18.6*		*25.1*		*10.5*		*82.5*
Propylthiouracil, 25 mg.															
E.J.	Graves' disease	2.4	+11.0	115.0	52.8	62.2	−45.9	46.5	−40.0	52.8	−23.3	18.4	− 9.2	.288	− 13.9
V.R.	Graves' disease	2.0	+28.0	403.0	119.0	284.0	−29.5	135.5	−10.0	77.4	−16.4	16.4	+ 7.3	.640	− 1.0
M.J.	Normal	1.2	−10.7	117.8	34.6	83.2	−29.4	24.1	−36.9	38.5	−69.1	18.0	− 3.9	.208	+131.6
A.W.	Normal	1.4	−13.8	78.0	30.2	47.8	−38.7	18.4	−47.8	29.6	−57.1	19.4	−26.8	.190	+ 68.3
	Mean		*+ 5.6*		*59.2*	*119.3*	*−35.9*		*−33.7*		*−41.5*		*− 8.2*		*+ 46.5*
	Std. Dev.		*19.6*		*41.1*	*110.8*	*8.0*		*16.4*		*25.5*		*14.2*		*67.4*

* See text for explanation of abbreviations.

the influence of propylthiouracil in each patient was expressed as a percentage reduction of the control binding rate. When this was done, a fairly uniform response was noted among patients in each dosage group. Increasing doses of propylthiouracil were associated with increasing effect, as shown by a greater percentage reduction in the control binding rate. There was no apparent difference in the sensitivity of the euthyroid and hyperthyroid binding mechanisms, since at each dose level no appreciable difference was noted in the effect obtained in the two groups.

Thyroidal Iodide Space (TIS): This measure of the ability of the thyroid gland to concentrate inorganic iodide, was not affected in any consistent manner by the administration of propylthiouracil. No difference in the response of the TIS could be noted between euthyroid and hyperthyroid patients, and between different dose levels of propylthiouracil.

Thyroidal Iodide Transfer Rate (T_T): Increasing doses of propylthiouracil produced increasing reduction in the thyroidal iodide transfer rate. No difference in the response of euthyroid and hyperthyroid individuals was noted. The reduction in transfer rate at each dose level was proportional to the reduction in protein-binding rate.

Plasma Radioiodide Disappearance Rate (k): The rate of disappearance of radioiodide from the plasma of hyperthyroid patients was decreased by propylthiouracil at all dose levels. In euthyroid patients, the disappearance rate decreased following doses of 100 mg. but increased following doses of 25 mg. Variable effects on the disappearance rate were noted in euthyroid patients receiving 50 mg.

Ultimate Thyroidal Uptake of Radioiodine (I_U): Propylthiouracil decreased the predicted value for the ultimate thyroidal uptake of radioiodine in all patients. In patients receiving the 100-mg. dose, and in thyrotoxic patients receiving either of the smaller doses, the percentage decrease in I_U was smaller than the percentage decrease in K or T_T. In euthyroid patients receiving either 25 or 50 mg. of propylthiouracil, in whom the plasma iodide disappearance rate increased following the administration of the antithyroid agent, the percentage reduction of I_U exceeded the percentage reduction in either T_T or K.

DISCUSSION

In intact animals and man, and in surviving slices of thyroid tissue, agents of the thiouracil series have been shown to interfere with the conversion of thyroidal iodide to hormone, but not with the ability of the thyroid gland to concentrate inorganic iodide.

It has been demonstrated elsewhere that the rate of transfer of plasma iodide to hormonal iodine (thyroidal transfer rate) is dependent upon the ability of the gland to concentrate inorganic iodide and to convert glan-

dular iodide to hormonal iodine (12). It might thus be expected that the administration of single doses of propylthiouracil to patients would result in a diminution in the rate of protein-binding of iodide and no diminution in the glandular concentration of inorganic iodide. As a result, the thyroidal transfer rate would be decreased. Since the total rate of removal of iodide from the plasma is dependent in part upon the thyroidal transfer rate, reduction in the latter function should diminish the rapidity with which radioiodide disappears from the plasma. Furthermore, the maximal accumulation of I^{131} in the thyroid glands of patients given propylthiouracil should also be diminished. The findings in the present study will be discussed in relation to these theoretical considerations.

The reduction in protein-binding activity which occurred in all patients given propylthiouracil is consistent with the known mechanism of action of this drug and supports the validity of the method by means of which the binding rate was calculated.

As might be expected from previous observations in animals and surviving tissue slices, calculated values of the thyroidal iodide space were not consistently or significantly reduced by the administration of single doses of propylthiouracil. As has been noted elsewhere, penetration of radioiodide into the thyroidal iodide space is largely represented in the iodine uptake curve by the rapid initial rise in the thyroidal content of I^{131}, occurring shortly after the administration of the tracer (12). Schultz and Jacobson, studying the effect of prolonged administration of propylthiouracil on the thyroidal accumulation of I^{131}, found that, as compared with the control tests, accumulated I^{131} was decreased but little during the early period following administration of the tracer (13). However, the magnitude of the decrease in thyroidal accumulation of I^{131} grew larger with the passage of time. Failure of propylthiouracil to alter the early accumulation of I^{131} is consistent with the belief that this segment of the radioiodine accumulation curve is largely representative of the iodide-concentrating function of the gland, which is not inhibited by propylthiouracil. The subsequent slope of the uptake curve is, however, in part a function of the rate of protein-binding of thyroidal iodide. Inhibition of this function by propylthiouracil would be expected to produce a progressively increasing reduction in the uptake of I^{131}, as compared with control tests (Fig. 2).

In animals and in surviving thyroid slices, inhibition of protein-binding is associated with augmentation of the ratio of radioiodide concentrations in the thyroid and its perfusate (14, 15). Failure to note a similar effect in the studies herein presented is not readily explained. In none of the present patients was organic binding completely inhibited, however,

The rate of disappearance of radioiodide from the plasma depends on the rapidity of removal of I^{131} by both the thyroid gland and by extrathyroidal

sites. Reduction in thyroidal function will thus diminish the exponential disappearance rate, provided the extrathyroidal removal of radioiodide is not concomitantly increased. When, as in hyperthyroidism, the thyroid contributes largely to the total removal of radioiodide, reduction in the function of the gland will markedly diminish the disappearance rate. When

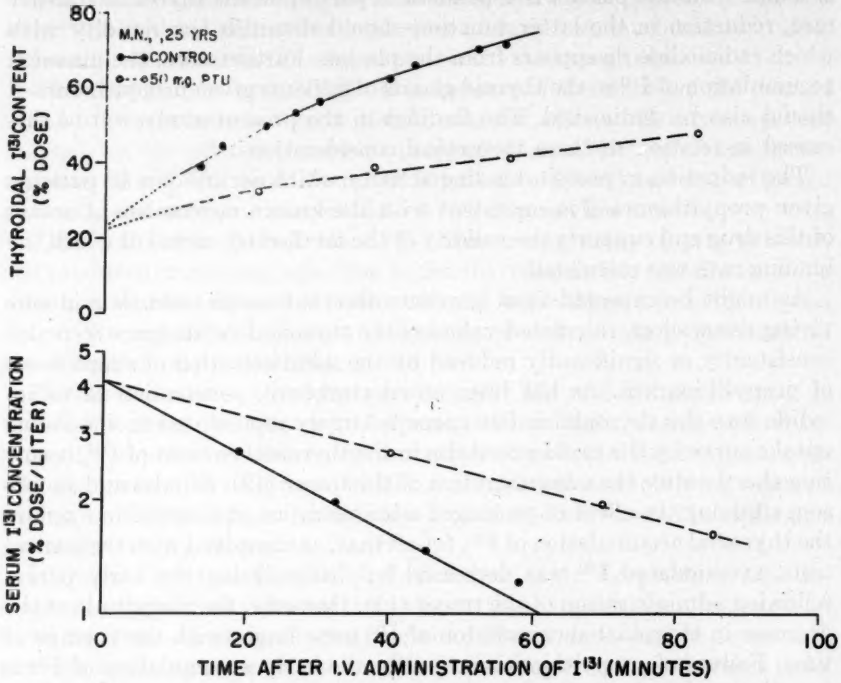
M.N., ,25 YRS
CONTROL
50 mg. PTU
THYROIDAL I131 CONTENT (% DOSE)
SERUM I131 CONCENTRATION (% DOSE/LITER)
TIME AFTER I.V. ADMINISTRATION OF I131 (MINUTES)

FIG. 2. Propylthiouracil in a dose of 50 mg. did not alter the magnitude of the rapid early accumulation of I^{131} or the extrapolated value (time = 0) of plasma I^{131} concentration. The subsequent slopes of both the thyroidal accumulation and plasma disappearance curves are, however, markedly diminished. Note that, as compared with the control test, the reduction in the thyroidal accumulation of radioiodine following the administration of propylthiouracil increases with the passage of time.

thyroidal removal of I^{131} is not rapid, reduction in glandular function will diminish the disappearance rate less markedly. Thus in thyrotoxic patients, the plasma radioiodide disappearance rate decreased following even the smallest dose of propylthiouracil. In euthyroid patients, a more variable response was noted. It will be recalled that the test performed following the administration of propylthiouracil frequently included some period during which extravascular mixing of injected iodide was incomplete. Prior to complete mixing of radioiodide, passage of this material from the plasma

into the remainder of its compartment of distribution will augment the disappearance rate (16). In euthyroid patients receiving the smaller doses of propylthiouracil, the relatively small reduction in the thyroidal component of radioiodide disappearance was overshadowed by the component contributed by specific radioactivity equilibration. This conclusion is substantiated by two findings. In such patients the increase in disappearance rate was calculated to be the result of increased extrathyroidal removal of radioiodide. Furthermore, following completion of mixing, curves of the plasma concentration of radioiodide uniformly displayed a less pronounced slope than that observed during the control test.

It has been repeatedly pointed out that the total accumulation of radioiodine within the thyroid gland is, for any level of thyroidal function, dependent upon the rapidity of removal of radioiodine from the plasma (17–19). The ultimate thyroidal uptake of radioiodine has been shown to be directly proportional to the thyroidal transfer rate, and inversely proportional to the plasma radioiodide disappearance rate (12). Thus, in the present study, those patients in whom the disappearance rate decreased under the influence of propylthiouracil demonstrated smaller reductions in ultimate uptake than in thyroidal transfer rate.

When radioactive iodine is administered to animals receiving propylthiouracil, the thyroidal content of I^{131} increases rapidly and then slowly declines (9). Such uptake curves are distinguished from the normal by their second or negative-slope phase. Previous authors have commented upon their failure to observe curves of this type in patients receiving propylthiouracil (13). An analysis of the factors controlling the slope of the radioiodine uptake curve serves to clarify the infrequency with which curves of this type are noted in man.

It has been demonstrated elsewhere that the slope of the radioiodine uptake curve may be represented by the following expression (12):

$$\text{TIS } I_{B_0} e^{-kt}(K - k)$$

where I_{B_0} = extrapolated (time = 0) concentration of I^{131} in plasma. The slope of the curve will be negative when k exceeds K, *i.e.*, when the rate of loss of radioiodide from the plasma, and hence from the thyroidal iodide space, exceeds the rate of transfer of radioiodide from the TIS to the glandular pool of organified iodine. When the two rates are equal the curve will display an early rapid rise, representing the rapid penetration of inorganic radioiodide into the thyroidal iodide space, followed by a curve which neither rises nor falls, the glandular content of radioiodidine remaining constant. Furthermore, since k is considerably larger during the period in which the tracer is mixing than after mixing is complete, it is possible for the thyroidal I^{131} uptake curve to display a negative slope followed

by a flat and then a positive slope as mixing occurs and is gradually completed. Each of these varieties of radioiodine uptake curve has been noted in the present study.

The "flat" uptake curve, obtained when k and K are equal, represents the boundary between the usual radioiodine uptake curve with a positive slope and the negative-slope curve just described. An analysis of the kinetic relationships present when k and K are equal will define the factors controlling the shape of the radioiodine accumulation curve.

As noted earlier (12), where

C_{ET} = extrathyroidal iodide clearance rate

IDS = iodide distribution space ($100/I_{B_0}$)

ETIDS = extrathyroidal iodide distribution space (IDS-TIS)

$$k = \frac{K\ TIS\ I_{B_0}}{100} + \frac{C_{ET}I_{B_0}}{100}.$$

If $k = K$,

$$K = \frac{K\ TIS\ I_{B_0}}{100} + \frac{C_{ET}I_{B_0}}{100}$$

$$K\left(1 - \frac{TIS\ I_{B_0}}{100}\right) = \frac{C_{ET}I_{B_0}}{100}$$

$$K = \frac{C_{ET}I_{B_0}}{100} \Big/ \frac{100 - TIS\ I_{B_0}}{100}$$

$$= C_{ET}I_{B_0}/(100 - TIS\ I_{B_0})$$

$$= C_{ET}/[(100/I_{B_0}) - TIS]$$

$$= C_{ET}/(IDS - TIS)$$

$$= C_{ET}/ETIDS$$

It is thus seen that k and K are equal when the numerical value of K is the same as the fraction of the extrathyroidal iodide distribution space cleared hourly by extrathyroidal sites. When mixing is complete, the principal extrathyroidal site of removal of iodide is the kidney. The quotient described here is therefore similar to, but not identical with the renal excretion rate as defined by Keating *et al.* (17). The slope of the radioiodine uptake curve is thus dependent on the relationship between the magnitude of the thyroidal iodide protein-binding rate and the rapidity with which the kidneys remove iodide from extrathyroidal tissues.

Since the renal rate of clearance of iodide is normally about 2 liters per hour (18), and since the ETIDS is approximately 20 liters, K must ordinarily be reduced to less than 0.1 (10 per cent/hour) in order for a negative-

slope accumulation curve to occur. This represents a reduction in the normal binding rate of approximately 90 per cent and, according to the present data, would require a single dose of more than 100 mg. of propylthiouracil. In hyperthyroid patients, in whom the binding rate is usually considerably increased (12), reductions in excess of 90 per cent and doses considerably in excess of 100 mg. would be required to reduce the binding rate to the requisite low level. Since doses in excess of 100 mg. of propylthiouracil are rarely used therapeutically, it is not surprising that negative-slope accumulation curves are rarely observed in patients undergoing therapy for Graves' disease (13).

The ability of the hyperactive thyroid gland to concentrate iodide is greater than normal (12, 20, 21). Since the concentration of inorganic iodide in the plasma of thyrotoxic patients appears to be at least as great as normal (15), the concentration of inorganic iodide within the hyperactive gland must be considerably increased. It is therefore of interest to note that the proportionate reductions of binding rate produced by single doses of propylthiouracil did not differ between the hyperthyroid and normal groups. This independence of substrate concentration is consistent with the hypothesis that propylthiouracil inhibits protein-binding of thyroidal iodide by direct, noncompetitive inhibition of the enzymes involved in this process (22).

A conventional log-dose *vs.* response plot of the average percentage reduction in binding rate showed a good rectilinear fit for the three dose levels employed (Fig. 3). From this plot, the average single dose of propylthiouracil necessary to reduce the binding rate by 50 per cent was found to be approximately 40 mg. Because of the relatively small number of patients studied, a formal statistical analysis of the dose-response relationship was not made. The data suggest, however, that the method herein employed may be applicable as a means of assaying the relative potency of other antithyroid agents.

The increased avidity of the thyrotoxic gland for iodine results from an increased glandular mass, an increased ability of each unit mass to concentrate inorganic iodide, and an increased capacity to oxidize thyroidal iodide and bind the resulting iodine to an organic moiety. It is reflected in an increased rate of transfer of iodide from the plasma into the glandular hormone pool. For any concentration of iodide in the plasma, the thyroidal transfer rate is a measure of the rate of manufacture of hormone. It is reasonable to assume that restoration of a euthyroid state requires return of the increased transfer rate to normal. This may be accomplished by reducing either of the constituent functions, TIS or the binding rate. Therapy with the commonly used antithyroid agents is directed toward reduction of the binding rate. It appears, however, that the greater the increase in binding

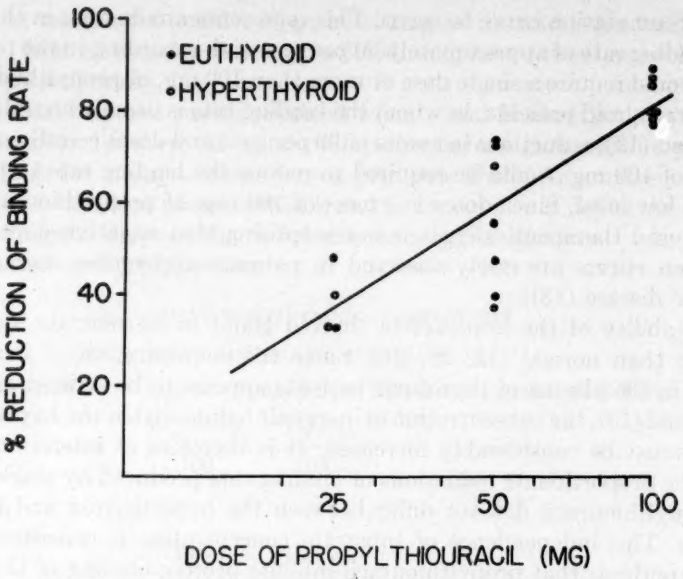

FIG. 3. Log-dose *vs.* response plot of percentage reduction in binding rate and dose of propylthiouracil. Approximately 40 mg. appears to be necessary to reduce the protein-binding rate by 50 per cent.

rate, the larger the dose of propylthiouracil necessary to restore this function to normal. Furthermore, when TIS is also increased, the binding rate must be reduced to subnormal levels, in order for the transfer rate to become normal. Thus it can be anticipated that patients with severe thyrotoxicosis, and especially those in whom TIS is markedly increased (generally patients with large thyroid glands), will be relatively refractory to therapy with this agent (2). In such patients, therapy might well be directed toward reduction of the TIS. This may be accomplished surgically, reducing the mass of the gland, or by means of agents such as thiocyanate or perchlorate, which reduce the capacity of the gland to concentrate inorganic iodide (23–25).

SUMMARY

In 10 euthyroid and 8 hyperthyroid patients, an evaluation has been made of the influence of propylthiouracil in single doses of 25, 50 and 100 mg. on the intrathyroidal mechanisms of hormone formation. No reduction of the ability of the gland to concentrate inorganic iodide was observed. A diminution in the thyroidal rate of transfer of iodide from the plasma into the glandular hormone pool occurred in all patients, and resulted from an inhibition of the rate of protein-binding of thyroidal iodide. The magni-

tude of the inhibitory effect was found to be proportional to the control level of the protein-binding rate and to the dose of antithyroid agent administered. Therapeutic implications of these findings have been discussed.

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RENAL CLEARANCE IN PATIENTS WITH MYXEDEMA

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DESPITE the considerable interest that exists in the cardiovascular abnormalities associated with myxedema, little can be found in the literature regarding renal function in this disorder. Corcoran and Page reported renal function studies on 2 patients suffering from myxedema, which demonstrated a reduction in renal blood flow, glomerular filtration, and tubular secretory capacity (1). The data of Beaumont and Robertson (2) indicate that urea clearance is depressed in myxedema, but that no change occurs in the concentrating ability of the kidneys.

The purpose of this paper is to present the results of glomerular filtration rate and effective renal plasma flow in 7 myxedematous patients.

METHOD

Specific clearance methods were used on 7 patients with untreated myxedema,[1] to Study the rate of glomerular filtration, the renal plasma flow, the filtration fraction, and the renal blood flow. Inulin clearances (C_{IN}) were measured to determine the rate of glomerular filtration, and measurements of plasma *p*-aminohippurate clearances (C_{PAH}) were used to determine the effective renal plasma flow. The filtration fraction (FF) is the calculated ratio of the clearances, C_{IN}/C_{PAH}. The renal blood flow (RBF) was calculated from the renal plasma flow and the hematocrit values.

The patients were hydrated, and were then given priming doses of inulin and PAH. Thereafter a constant intravenous drip of inulin and PAH solutions in a 0.9 per cent solution of sodium chloride (saline) was maintained. Urine specimens were collected from indwelling catheters by means of saline and air washing, as suggested by Goldring and Chasis (3). Alkali-stable inulin determinations were carried out according to the method of Little (4), and the PAH determinations by the method of Bratton and Marshall (5). The values reported are the means of three clearance periods.

MATERIAL

Case 1. This patient was a 52-year-old white woman rendered myxedematous by the use of radioactive iodine for the treatment of a malignant lesion of the thyroid gland. There was no previous history of hypertension or renal disease. A Fishberg concentration

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[1] The diagnosis of myxedema was established clinically and substantiated by the determination of the basal metabolic rate, the blood cholesterol level and, in some instances, by the blood protein-bound iodine level.

test performed at the time of the clearance studies revealed a maximum specific gravity of 1.029, and a phenolsulfonphthalein test showed 31 per cent excretion in fifteen minutes.

Case 2. This 50-year-old white woman had myxedema of six years' duration. There was no previous history of renal disease, but the patient's blood pressure was elevated to 150 systolic and 100 diastolic. Routine urinalysis showed no abnormality, and a specific gravity of 1.021.

Case 3. This white woman, aged 40, had myxedema resulting from a subtotal thyroidectomy performed two years prior to the renal function studies. There was no history of renal disease or hypertension. A routine urinalysis gave normal findings.

Case 4. This patient was a 38-year-old white woman with myxedema of eight years' duration. There was no history of hypertension or renal disease. Upon routine analysis the urine had a specific gravity of 1.025, and contained no albumin or abnormal microscopic elements.

Case 5. This patient, a 65-year-old white woman, gave a history suggestive of the insidious onset of myxedema many years prior to the renal clearance studies. There was no history of hypertension or renal disease. Results of a routine urine examination were entirely normal.

Case 6. This 66-year-old white man had postoperative myxedema of six months' duration. Although there was no history of renal disease, the patient for several years had had a labile blood pressure, fluctuating between 120 and 180 systolic and 80 and 110 diastolic. A diagnosis of arteriosclerotic heart disease had been made, but there was no evidence of cardiac decompensation.

Case 7. This patient was a 59-year-old man with myxedema of eleven years' duration. A previous diagnosis of arteriosclerotic heart disease and hypertension had been made, and the highest recorded blood pressure reading was 200 systolic and 110 diastolic. There was no history of renal disease. A phenolsulfonphthalein test made prior to the clearance studies showed only 5 per cent excretion in fifteen minutes, and a Fishberg concentration test showed a maximum specific gravity of 1.016.

RESULTS

The values for specific renal functions of the 7 patients are given in Table 1.

In the 5 females the average values for inulin clearance, PAH clearance, and filtration fraction, corrected to 1.73 sq. M., were 61.7, 256 and 0.264 respectively, compared with Homer Smith's (6) normal values for women of 108.8, 592 and 0.194. The average renal blood flow for 4 of these patients was 442 ml. per minute.

In the 2 men the mean values for inulin clearance, PAH clearance, and filtration fraction, corrected to 1.73 sq. M., were as follows: 67.9, 247 and 0.276, compared with normal values for males of 124.1, 654 and 0.192 (6).

TABLE 1. AVERAGE RENAL CLEARANCE VALUES IN PATIENTS WITH MYXEDEMA

Case	Sex	*C_{IN} ml./min.	*C_{PAH} ml./min.	RBF ml./min.	FF
1	F	58	340	516	0.186
2	F	93	330	502	0.280
3	F	75	315	523	0.248
4	F	40	153	228	0.260
5	F	42	299	—	0.142
6	M	86	250	641	0.346
7	M	49	244	387	0.206

* Corrected to 1.73 sq. M. surface area.

In these 2 cases the values for renal blood flow were 641 and 387 ml. per minute.

DISCUSSION

As indicated in Table 1, renal function studies in these 7 patients with myxedema demonstrated a trend toward a diminution in the glomerular filtration rate and renal plasma flow. The magnitude of this trend may be seen in Table 2, in which is shown the distribution frequency of our data with respect to normal values obtained by Smith (6). In 5 of the 7 patients the inulin clearance was below the range of the mean minus two standard deviations (2½ per cent probability level). The other 2 were below the range of the mean minus one standard deviation (between the 2½ and 16 per cent probability levels). Studies based on the clearance of PAH indicated that in 3 patients the renal plasma flow was below the range of the mean minus two standard deviations (2½ per cent probability level), and in the other 4 was below the range of the mean minus one standard deviation (between the 2½ and 16 per cent probability levels). The filtration fraction was more variable, and no clear pattern was evident. These findings are

TABLE 2. DISTRIBUTION FREQUENCY OF THE 7 CASES IN COMPARISON WITH THE NORMAL VALUES OF SMITH* (6)

	†$<\bar{X}-2\sigma$	$<\bar{X}-1\sigma$	$\bar{X}\pm1\sigma$	$>\bar{X}+1\sigma$	$>\bar{X}+2\sigma$
C_{IN}	5	2	—	—	—
C_{PAH}	3	4	—	—	—
FF	—	1	2	2	2

* Females: $C_{IN}=108.8$, σ 13.5; $C_{PAH}=592$, σ 153; FF=0.194, σ 0.039
Males: $C_{IN}=124.1$, σ 25.8; $C_{PAH}=654$, σ 163; FF=0.192, σ 0.035

† $\bar{X}$ = mean value; σ = standard deviation of data.

in agreement with the observations of Corcoran and Page (1).

Unfortunately, we were not able to restudy these individuals after the administration of desiccated thyroid.

SUMMARY

Renal clearance measurements of inulin and *p*-aminohippurate were carried out in 7 patients with myxedema. There was demonstrated a reduction in the glomerular filtration rate, the renal plasma flow, and the renal blood flow. The values for the filtration fraction were variable.

Addendum

Since this paper was submitted for publication, another article on the subject has been published in this journal (Hlad, C. J. and Bricker, N. S.: Renal function and I^{131} clearance in hyperthyroidism and myxedema, *J. Clin. Endocrinol. & Metab.* **14:** 1539 (Dec.) 1954).

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THE DIPHASIC CHARACTER OF THYROIDAL I^{131} CLEARANCE*

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MOST investigators have assumed the thyroidal I^{131} clearance to be constant (1–6). Oddie (7), however, does not base his excellent discussion of the dynamics of iodine metabolism on this presumption, and a recent analysis by Wollman (8) predicts that the clearance is larger in the early stages and decreases as a tracer study progresses. The present investigation was undertaken in order to gain further information concerning this general problem.

METHODS

Fifteen healthy young adults were given 200 microcuries of I^{131} orally or intravenously. Thyroidal, calf and urinary radiations, as well as total I^{131} and labeled protein- bound iodine (PBI^{131}) in the blood were determined at $\frac{1}{2}$, 1, 2, 3, 6, 12, 24, 48, 72 and 96 hours, by techniques to be described elsewhere (9, 10).

The results were corrected for extrathyroidal radioactivity by extrapolating the regression lines obtained from plotting, (a) the ratio of counts over the neck to counts over the leg, (b) the logarithm of this ratio, (c) the ratio of counts over the neck to the total serum radioactivity, and (d) the logarithm of this ratio. The calculated extrathyroidal radioactivity by all four techniques was similar, but that obtained from the logarithm of the neck-to-serum ratio (d), showed the greatest constancy and was therefore employed in this study to derive the correction factor for extrathyroidal radioactivity and thus, by difference, the true thyroidal radioactivity.

The percentage of the administered dose in the thyroidal fraction at any time was ascertained by adding the calculated thyroidal radioactivity to that already secreted by the thyroid gland into serum (PBI^{131} concentration times extracellular volume (11))

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and the clearance was determined by dividing changes in this fraction by the mean blood iodide value (2). In this way seven to nine different clearance values were obtained during the course of each study.

RESULTS

Thyroidal iodide clearances were found to be constant during Phase A (2–12 hours), after which 13 of 15 subjects demonstrated a decreased clearance (Phase B) which remained constant as long as measurements were accurate (24–72 hours) (Table 1). The mean clearance rate for the

TABLE 1. EARLY (A) AND LATE (B) MEAN THYROIDAL I^{131} CLEARANCES

Case No.	Phase A (L./hr.)	Phase B (L./hr.)	Change (%)
1	1.21	.55	−55
2	1.20	.74	−38
3	1.72	1.18	−31
4	1.07	.65	−44
5	1.25	.60	−52
6	.76	.33	−57
7	1.24	1.42	+15
8	1.09	.81	−26
9	1.05	.89	−15
10	.83	.69	−17
11	.56	.30	−46
12	1.17	.66	−44
13	.65	.70	+ 8
14	.73	.41	−44
15	.90	.38	−58
Mean (entire group)	*1.03*	*.79*	*−23*
Mean (excluding 7 & 13)	*1.04*	*.63*	*−39*

entire group during Phase A was 1.03 liters per hour, whereas during Phase B it was 0.79 liter per hour, a fall of 23 per cent. In the 13 subjects who showed a decrease in the clearance rate, the mean fall was 39 per cent —from 1.04 to 0.63 liters per hour.

The difference between the serum total I^{131} and PBI^{131} concentrations was assumed to be the serum iodide level. When the serial concentrations of serum radioiodide of a euthyroid individual were plotted semilogarithmically after intravenous administration, as many as four separate phases were noted (Fig. 1). Following oral administration the first phase, possibly

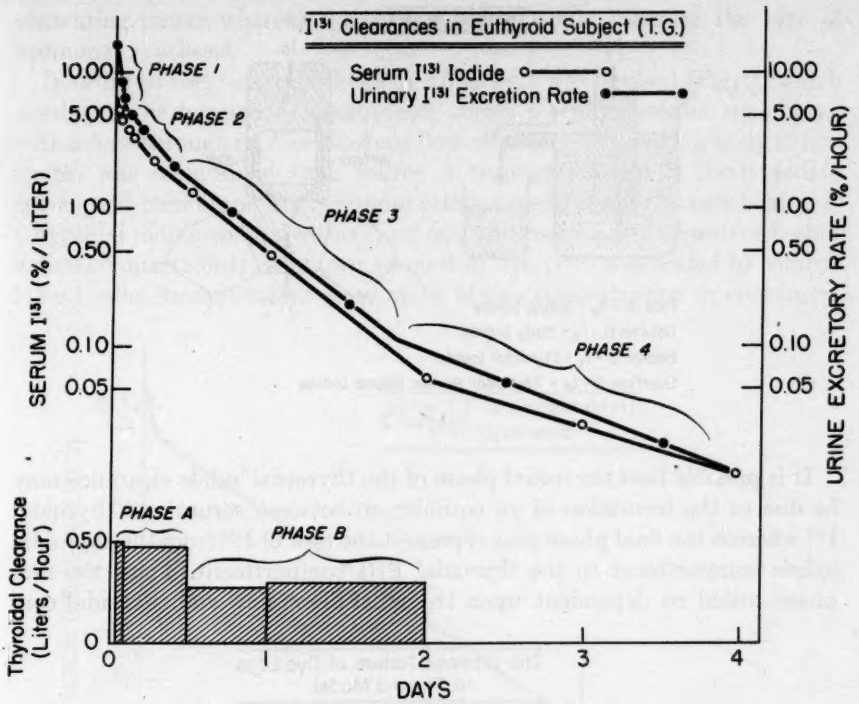

FIGURE 1

due to dilution of I^{131} through the body space, was generally obscured. In many patients, the fourth phase, probably related to recovery of iodide by endogenous breakdown of PBI^{131}, did not form a straight line.

When the urinary excretion rates (percentage of dose per hour) were plotted semilogarithmically, the same four phases were noted in most individuals (Fig. 1). Both slope rates are similar after the first hour and the two curves are parallel, indicating that the urinary iodine clearance is constant.

DISCUSSION

The occurrence of a diphasic thyroidal I^{131} clearance is indicated by the direct data (Table 1) and is independently supported by the serum radioiodide disappearance and urinary radioiodide excretion rates (Fig. 1). The change from Phase A to Phase B in thyroidal clearance coincides with the shift from Phase 2 and 3 in both blood and urine, suggesting that the clearance data are valid and that at least a partial explanation for the shift is on this basis.

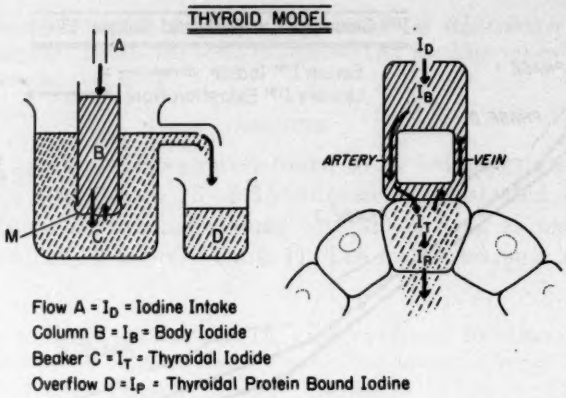

FIGURE 2

It is possible that the initial phase of the thyroidal iodide clearance may be due to the formation of an equilibrium between serum and thyroidal I^{131} whereas the final phase may represent the flow of I^{131} from the thyroidal iodide compartment to the thyroidal PBI compartment. Thus, the first phase would be dependent upon thyroidal blood flow and thyroidal con-

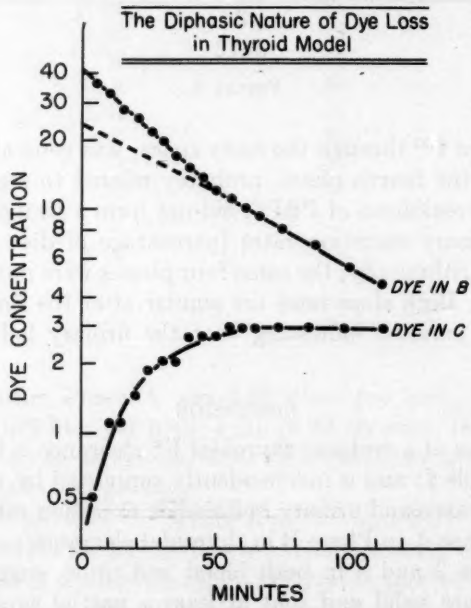

FIGURE 3

centrating power whereas the second phase would measure the rate of hormonal synthesis.

In order to test this hypothesis an apparatus was devised (Fig. 2) which combined the features of equilibrium across a semipermeable membrane with a flow through it. A continuous flow of water (I^{127} entering body iodide space) was maintained from source A through column B (body iodide space) and membrane M (thyroidal cellular membrane) through beaker C (thyroidal iodide compartment) and out into receptacle D (protein-bound iodine compartment). A known amount of dye (I^{131}) was added to column B and serial measurements were made of dye concentration in containers

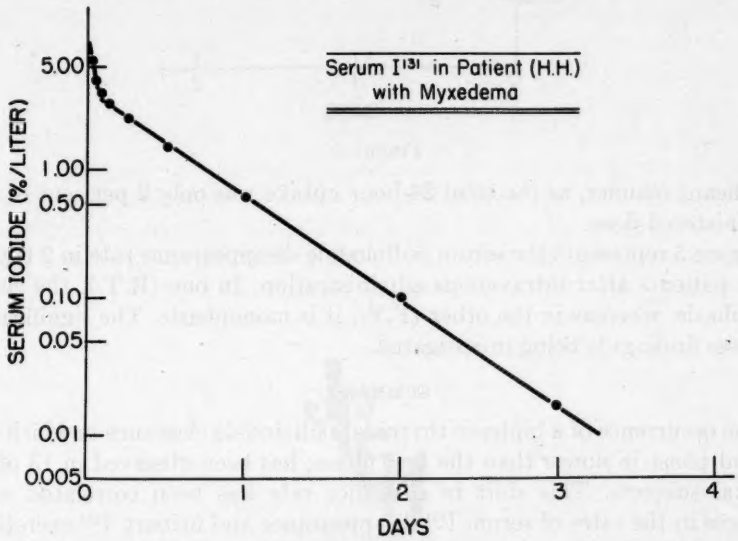

FIGURE 4

B and C. The results of this experiment are shown in Figure 3, from which it may be seen that the loss of dye from column B (body iodide space) is diphasic and that the dye concentration in beaker C (thyroidal iodide compartment) comes to equilibrium at the time of the shift.

Thyroidal iodide clearance studies have been performed in patients with thyroid disease. The serum radioiodide disappearance rate in myxedema after intravenous administration is shown in Figure 4 and is as described by Brownell (5). After the initial phase of dilution through the body iodide space, the disappearance rate is constant, indicating absence of the thyroidal factors noted in normal subjects. The thyroidal iodide clearance on this patient was so low that it could not be determined in a statistically

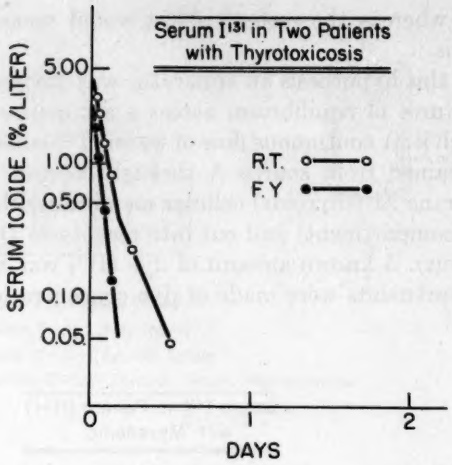

FIGURE 5

significant manner, as the total 24-hour uptake was only 2 per cent of the administered dose.

Figure 5 represents the serum radioiodide disappearance rate in 2 thyrotoxic patients after intravenous administration. In one (R.T.), the curve is diphasic, whereas in the other (F.Y.) it is monophasic. The significance of these findings is being investigated.

SUMMARY

The occurrence of a biphasic thyroidal radioiodide clearance in which the second phase is slower than the first phase, has been observed in 13 of 15 normal subjects. This shift in clearance rate has been correlated with changes in the rates of serum I^{131} disappearance and urinary I^{131} excretion. An apparatus has been constructed and tested, for use in correlating these findings with an initial phase of equilibrium and a final phase of hormonal synthesis.

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PROTEIN-BOUND IODINE BY ALKALINE INCINERATION AND A METHOD FOR PRODUCING A STABLE CERATE COLOR

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THE proposed method controls the reaction of the iodine monochloride arsenious sulfato-ceric acid catalysis in the quantitative estimation of microgram amounts of iodine. This is accomplished in four steps. First, conditions are adjusted so that the ceric ions in each sample are inactive. Second, conditions are changed, allowing the reaction to proceed simultaneously in each specimen for a determined time interval. Third, the catalysis is halted abruptly. Fourth, a stable cerate compound is produced, so that transmittance measurements can be made without color drift.

The proposed method prevents iodine loss from solution during analysis by keeping it stabilized in alkaline medium. Conditions are not made acid until the reaction-controlled introduction of ceric ion.

Potassium iodide standards are prepared in 4.0 N sodium carbonate, in which the iodine is kept in a reduced state, with no loss, for periods of more than one year at room temperature. Working standards and blanks are carried through each step of analysis along with test samples and are utilized, without introducing variables, as controls. It is possible to include several standards with each run, since the method of color development does not place limits on the number of specimens that constitute a batch.

Recently, the authors have included thyroxine standards as additional controls, particularly for checking the incineration temperature. It was found in a limited number of experiments that loss of iodine occurred with a mixture of thyroxine and serum, but not with potassium iodide, when the incineration temperature was increased to a pyrometer indicator reading of 680° C.

The authors have observed that 4.0 N sodium carbonate absorbs iodine. It follows, that solutions prepared at different times may produce variable blanks. Therefore, all working standards are diluted with aliquots of the same solution used in the blanks and test samples. Solutions are renewed when the blank reaches a value of 1.0 microgram per 100 cc.

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METHOD

Reagents

Reagents: All solutions are aqueous, all chemicals of highest purity.

Water: Water used for reagents, or for such purposes as cleaning glassware is iodine-free. If necessary, the final distillation is from sodium hydroxide, using an all-glass still.

Zinc sulfate, 10 per cent: 100 grams of $ZnSO_4 \cdot 7H_2O$ per liter of solution.

Sodium hydroxide, 0.5 N: 20 grams of NaOH per liter of solution. NOTE: 10.8 to 11.2 cc. of this solution should produce a permanent pink color when titrated into 10.0 cc. of 10 per cent zinc sulfate, with phenolphthalein.

Sodium carbonate, 4.0 N: 212 grams of anhydrous Na_2CO_3 per liter of solution.

Hydrochloric acid, 2.0 N: 175 cc. of the concentrated acid (S.G., 1.18) per liter of solution.

Basic arsenic reagent:

(A.) Stock solution: dissolve 7.6 grams of As_2O_3 and 4.0 grams of NaOH in a small amount of water and dilute to 100 cc.

(B.) Working arsenic reagent: add 90 cc. of water to 10 cc. of (A.) and mix.

NOTE: Although arsenite is slowly oxidized to arsenate by air under alkaline conditions, the foregoing solutions are stable with respect to the reaction described in this paper.

Sulfuric acid, 7.0 N: 200 cc. of the concentrated acid (S.G., 1.84) per liter of solution.

Ceric ammonium sulfate: Dissolve about 5 grams of the salt in a liter of 7.0 N sulfuric acid. Exactly 1.0 cc. of this solution, mixed with 5.5 cc. of water in a colorimeter tube, should read about 160 in the Klett-Summerson colorimeter, using water as the zero reference point, with a blue #42 light filter. If the color is too weak, add more solid ceric ammonium sulfate; if too strong, dilute with 7.0 N sulfuric acid.

Brucine sulfate, 1 per cent: 1 gram per 100 cc. of solution.

Standard potassium iodide:

(A.) Stock standard 1: Dilute 130.8 mg. of potassium iodide to a liter with 4.0 N sodium carbonate. This solution contains 100 micrograms of iodine per cc.

(B.) Stock standard 2: Dilute 10.0 cc. of (A.) to 100 cc. with 4.0 N sodium carbonate. This solution contains 10 micrograms of iodine per cc.

(C.) Working standard 1: Dilute 1.0 cc. of (B.) to 100 cc. with 4.0 N sodium carbonate. The carbonate used in this dilution is an aliquot from the solution that will be used in the blanks and test samples. This iodide solution contains 10 micrograms of iodine per 100 cc.

(D.) Working standard 2: Dilute 2.0 cc. of (B.) to 100 cc. in the same way as in (C.). This solution contains 20 micrograms of iodine per 100 cc.

Apparatus

Muffle furnace: Hotpack, Model No. 7069, with indicating pyrometer.

Hot air oven: Electric; constant temperature to 90° C.

Water bath: Electric; constant temperature, 37° C.

Centrifuge: International, Size 1, Type SB with 50-cc. cups.

Photoelectric colorimeter: Klett-Summerson with an external, mirror-type galvanometer (Leeds Northrup 2420 C) replacing galvanometer in colorimeter, and with a non-fluctuating power supply.

Centrifuge tubes: 50-cc. capacity without spout, heavy wall—Rockefeller Institute for Medical Research, under their designation, "N-1"; or A. H. Thomas No. 3121-A.

NOTE: This tube was selected from a number of types because of its remarkable ability to withstand the conditions of test. Many of these tubes are still in service after more than 100 runs.

Procedure

1. Into the 50-cc. centrifuge tube pipette 7 cc. of water. While stirring with a thin glass rod, add 1.0 cc. each, of serum, 10 per cent zinc sulfate, and 0.5 N sodium hydroxide.

2. Mix gently, but completely. Allow to stand for five minutes. Remove glass rod, after rinsing with about 1 cc. of water, and store rod in a test tube. The rod is used for the particular sample throughout the analysis.

3. Pack protein precipitate in centrifuge. Discard clear, colorless, supernatant liquid.

4. With stirring rod, mix packed protein and spread along inside lower fourth of tube. This enables the analyst to carry out step 5 with ease.

5. With 10 cc. of water in a pipette, wash down the sides of the tube into the protein. With the rod, mix protein and water so that no lumps remain, and all the protein is in uniform suspension. Rinse rod.

6. Pack precipitate in centrifuge. Discard clear, colorless, supernatant liquid.

7. Repeat steps 4, 5 and 6.

8. Add exactly 1.0 cc. of 4.0 N sodium carbonate to sample, and mix completely with glass rod. Rinse rod with about 1 cc. of water. Dry protein carbonate mixture in hot air oven (85° to 90° C.) overnight.

NOTE: The blanks and the standards do not contain serum, but otherwise the procedure is the same. In the case of standards, in step 8, the volume of the standard plus the volume of sodium carbonate equals 1.0 cc. It is our practice to include standards with values of 3.5 micrograms per 100 cc. and 7.0 micrograms per 100 cc. This is accomplished by using 0.35 cc. of working standard #1 with 0.65 cc. of 4.0 N sodium carbonate solution for the 3.5-microgram standard; and 0.70 cc. of working standard #1 with 0.30 cc. of sodium carbonate for the 7.0 -microgram standard.

9. Place the centrifuge tubes containing the dried powder, including samples, standards and blanks, in the muffle furance. Incinerate for two and a half hours at a pyrometer indicator reading of 570° C. ($\pm 10°$ C.).

10. To the cooled residue, add exactly 1.0 cc. of 2.0 N HCl, washing down the sides of the tube. Mix well with stirring rod, breaking up the small amount of carbon residue, silica and zinc salts.[1]

[1] Barker *et al.* (1) produce an acid medium at this point. They mention the importance of waiting until the odor of hydrogen sulfide is dissipated, to avoid precipitation of ceric

11. Add 1.5 cc. of water and mix. Add 10.0 cc. of water with a pipette, rinsing the rod, and washing down the sides of the tube. Mix well, and allow to stand for a few minutes to drain. Mix again. Remove stirring rod.

12. Pack down in centrifuge. Pipette off 10 cc. of the supernatant liquid, and put two 5.0-cc. portions into colorimeter tubes.

13. Place duplicates of test samples, standards and blanks in a suitable rack. Add 0.5 cc. of working arsenic reagent to each tube.

14. Mix the contents of each tube well. All mixing of solutions, from this point on, must be carried out by some technique that avoids both contamination and solution loss. The authors prefer the "flipping technique" described by Barker *et al.* (1). This is performed by grasping the colorimeter tube close to the open end, between thumb and index finger, and inverting the tube rapidly by raising it over one's head, and rapidly returning it. The authors have been able to accomplish complete mixing with five such flips.

15. Place rack, containing entire run, in ice-water bath in refrigerator for about ten minutes, to cool samples to 4° C. or lower. (For added convenience and increased speed, a chest-type freezer, into which a pipette can easily be introduced may be substituted for the ice-water bath and refrigerator.)

16. Remove ice-water bath, containing rack, from refrigerator. Add, without removing tubes from bath, exactly 1.0 cc of ceric ammonium sulfate to each tube. (Ceric solution has been previously cooled, and is kept in ice-water bath during pipetting.)

17. One tube at a time, give five rapid flips to each tube, and replace the tube in its respective position in the rack.

18. Place the rack in a water bath at 37° C. for exactly fifteen minutes. During this time, return the ice-water bath to the refrigerator and add ice if necessary.

19. After fifteen minutes, remove rack from heated water bath, and place immediately in ice-water bath in refrigerator for about ten minutes, in order to cool samples to 4° C. or lower.

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sulfide when ceric sulfate is added later in the analysis. Under the conditions of the test described here, where the solution is still alkaline after the addition of 1.0 cc. of 2.0 N hydrochloric acid, it has not been found necessary to take this precaution. No precipitation or other interference has been observed with the addition of the ceric sulfate. No sulfide odor is evident after this addition, at which time the medium is acid. Much of the sulfide in the alkaline solution, after the addition of the hydrochloric acid, exists as insoluble zinc sulfide. There is good possibility that the small amount of sulfide that may not be precipitated as zinc sulfide is either dissipated rapidly as hydrogen sulfide, or is converted to sulfate in alkaline medium, and could serve as a further protection for keeping the iodine in a reduced state with the following reaction:

$$S^{=}+4I_2+8OH^{-}\rightarrow SO_4^{=}+8I^{-}+4H_2O$$

20. Remove ice-water bath, containing rack, from refrigerator. Add, without removing tubes, 0.5 cc. of brucine sulfate solution to each tube. (Brucine solution has been previously cooled.)

21. Repeat step 17.

22. Remove rack from ice-water bath, and leave at room temperature until condensation is gone, or place in water bath at 37° C. for five minutes.

23. The color is stable, and can be read any time up to at least twenty-four hours, providing precautions are taken to prevent solution evaporation. However, as with most methods, the authors recommend that a precise time schedule be set up.

24. Read in a Klett-Summerson colorimeter, using water as the zero reference point, with a blue #42 light filter.

25. Calculation of results:[2]

$$\frac{\text{Reading of blank minus reading of sample}}{\text{Reading of blank minus reading of standard}} \times \text{micrograms I in std/100 cc.}$$

$$= \text{micrograms of PBI in 100 cc. of serum.}$$

Example: Blank reads 450

Standard (3.5 micrograms/100 cc.) reads 350

Unknown reads 303

$$\frac{450 - 303}{450 - 350} \times 3.5 = 5.1 \text{ micrograms/100 cc. in unknown}$$

Preparation of standard curve

The cerate color formed with brucine sulfate follows Beer's law, in the entire range of valid optical densities, from 0.2 to 0.7. It is for this reason that the foregoing calculations can be made. However, if the analyst pre-

[2] Although the brucine cerate color follows Beer's Law, considerable work was required to adjust conditions so that the catalysis proceeded at a rate to produce the curve shown in Figure 1. Different lots of ceric sulfate may vary in moisture content; and since the same scale reading on two Klett-Summerson instruments may not represent the same optical density, it may be necessary to adjust the cerate solution to a colorimeter reading somewhat different from that given in the method. Also, in order to compensate for differences in the other reagents, it may be necessary to lengthen, or shorten, the time of reaction in the 37° C. water bath. If it is not practical for the analyst to make this variation to suit the particular conditions that exist in his laboratory so that the reaction rate is controlled as shown in Figure 1, a straight-line curve will not be obtained, and the method of calculation of results will not be valid. An alternate method of obtaining results, with possible loss of accuracy, must be used. This is accomplished by referring to the curve and making adjustments, if necessary, in consideration of the readings of the blanks and standards of the particular run, and obtaining the result in micrograms of iodine per 100 cc.

fers (although it is not recommended), he can prepare a standard curve by the proper addition of working standards Nos. 1 and 2, with appropriate amounts of 4.0 N sodium carbonate, to washed zinc hydroxide, as indicated in step No. 8 of the procedure. The authors strongly urge that controls be carried out with each run, and do not recommend the use of a curve for obtaining results. Figure 1 shows a curve, in which standards with values from 0.0 to 14.0 micrograms of iodine per 100 cc. are plotted on coordinate paper against the respective values obtained by subtracting the respective

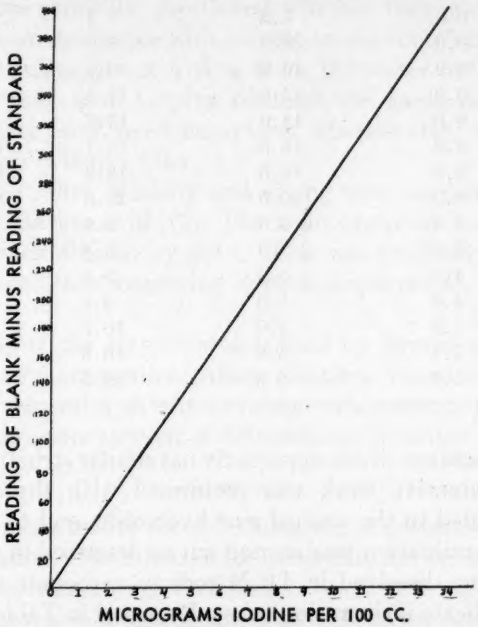

FIG. 1. Standard curve showing relationship between iodine content and values obtained by subtracting respective Klett-Summerson readings of standards from reading of blank (blank in this case read 450).

colorimeter readings from the reading of the blank. The blank, in this case, gave a reading of 450.

RESULTS

The average value for serum protein-bound iodine obtained on 53 euthyroid individuals was 5.0 micrograms per 100 cc., with a range of 3.3 to 7.4. This is in good agreement with the results of Barker *et al.* (1), McClendon and Foster (2), and Brown *et al.* (3).

As a test of the reliability of the method in determining known amounts

TABLE 1. RECOVERY OF IODINE IN THYROXINE ADDED TO WASHED ZINC HYDROXIDE AND WASHED PRECIPITATED PROTEIN

Test No.	Iodine (micrograms per 100 cc.)			
	In sample	Added	Found	
1	0.0	2.0	2.0	
2	0.0	3.5	3.5	
3	0.0	5.0	5.2	
4	0.0	7.0	7.3	
5	0.0	8.0	8.0	
6	0.0	10.0	10.3	
7	0.0	12.0	11.8	
8	0.0	14.0	13.6	
9	0.0	16.0	16.1	(half quantity)
10	0.0	18.0	18.3	(half quantity)
11	0.0	20.0	20.6	(half quantity)
12	3.3	5.0	8.4	
13	3.8	5.0	8.8	
14	4.1	5.0	9.5	
15	4.6	5.0	9.9	
16	5.0	5.0	10.2	
17	5.1	5.0	10.0	
18	5.3	5.0	10.5	

of iodine in a substance which apparently has similar structure to protein-bound iodine, extensive work was performed with thyroxine. Known amounts were added to the washed zinc hydroxide, and to washed serum protein. The determination was carried out as described in the procedure, with the thyroxine dissolved in 4.0 N sodium carbonate. Each analysis was made in triplicate incineration tubes. Reported in Table 1 are average representative results, which demonstrate satisfactory recovery. It may be noted in Table 1, that tests Nos. 9, 10 and 11 were performed with half quantities. This was accomplished by using 2.5 cc. of sample from its incineration tube, and 2.5 cc. of blank, from its incineration tube.

DISCUSSION

In the determination of protein-bound iodine by alkaline incineration, a reaction has been introduced, with which it is now possible to obtain a stable cerate color that follows Beer's law. Our initial experiments involved an attempt to remove or tie up the arsenious ion, and thereby stop the reaction. This was not successful, so the line of attack was shifted to the ceric ion. This was accomplished by introducing a brucine derivative into a cooled system, when the ceric ion was completely dormant with respect to

its activity in the catalysis. The brucine portion of the molecule combined with the ceric ion, removing it from the system and forming a stable compound.

The method makes it possible to complete many more specimens in one run than with previously published techniques. Limits are set, not by the method, but by the number of the personnel and the type of equipment available. In addition, the brucine reaction enables the analyst to attain a higher degree of accuracy. With the use of previously published techniques, the authors have seriously questioned whether they were achieving reproducibility in every sample with respect to the time intervals in removing colorimeter tubes one at a time from the water bath and replacing them; whether they were keeping constant the speed of pipetting ceric ammonium sulfate; and, most important, whether they were duplicating readings of a rapidly fading color.

Protein precipitation, washing and ashing were carried out essentially as described by Barker *et al.* (1). The main exception was in the ashing temperature, which differed by 30° C. This was probably due to a variation in the temperature measuring devices employed in the two laboratories.

Use was made of the technique described by Brown *et al.* (3) for the preparation of ceric ammonium sulfate solutions, based on transmittance readings rather than on a weight-to-volume relationship, in consideration of the variable moisture content of different lots of cerium salts.

SUMMARY

A method is presented for the determination of protein-bound iodine by alkaline incineration. Conditions of the procedure are designed to promote reproducibility by eliminating variables.

Introduced is a reaction that produces a stable cerate color which follows Beer's law in the entire range of valid optical densities, and which does not place limits on the number of samples that constitute a test run.

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Teaching Clinic

COMPLICATIONS IN THE MANAGEMENT OF CUSHING'S SYNDROME, INCLUDING ANAPHYLACTIC REACTION TO INTRAVENOUS ADRENOCORTICOTROPIN AFTER SUBTOTAL ADRENALECTOMY*

RENEWED interest in the definitive medical and surgical therapy of Cushing's syndrome has been stimulated by two major therapeutic advances. The first of these is the general availability of adequate agents for the hormonal replacement therapy of spontaneous or induced adrenal insufficiency. The efficacy of cortisone in this disorder is now well known (1). Secondly, techniques of adrenal surgery have been developed which allow excellent exposure and removal of the adrenal glands with a minimum of surgical risk to the patient (2). Adrenalectomy in patients with prostatic cancer (3) or essential hypertension (4) is relatively uncomplicated. However, this same procedure in a case of Cushing's syndrome presents numerous problems of grave import to the patient. Postoperatively there may occur a poorly understood series of complications, including repeated episodes of adrenal crisis, marked vasomotor instability and episodes of nausea and hypotension.

It is the purpose of this report to present the case history of a 40-year-old woman with Cushing's syndrome and to describe the series of complications that followed subtotal adrenalectomy. In particular, a severe anaphylactic reaction to ACTH was observed in this patient. This unusual phenomenon stimulated studies concerning the characteristics of this reaction.

CASE REPORT

S.L., female, aged 40.

First admission (Sept. 27–29, 1951)

This patient was admitted to the metabolism service after the diagnosis of Cushing's syndrome had been made by her private physician.[1] Seven years previously, during her last pregnancy, hypertension was discovered. Her blood pressure at that time was 240/160 mm. Hg and on subsequent determinations remained at this level. For six

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[1] We are grateful to Dr. Fred Clausen for referring this patient to us.

years the patient had had frequent headaches and epistaxes. Her menstrual periods did not recur after the last pregnancy. During the four years prior to admission there had been progressive obesity, the weight having increased from 140 to 225 pounds. Her face had become noticeably rounder. Weakness was a prominent symptom and the patient bruised easily. The past history and review of systems were not remarkable.

Physical examination: B.P. 290/180 mm.Hg, pulse 100, temperature 98° F. and respirations 20. There was marked truncal obesity with large fat pads over the cervicodorsal vertebrae and in the supraclavicular areas. The face was plethoric and moderate

TABLE 1. ADMISSION LABORATORY DATA ON PATIENT S.L.

Volume of packed red blood cells	47 ml./100 ml.
White blood cell count	10,400/cu. mm.
Differential:	
Polymorphonuclear neutrophils	79%
Juvenile neutrophils	2%
Basophilic neutrophils	1%
Lymphocytes	18%
Direct eosinophil count	22, 12, and 9 per cu. mm.
Urinalysis	Normal findings
Urinary concentration tests	Normal findings
Phenolsulfonphthalein excretion	Normal
Blood urea nitrogen	15 mg./100 ml.
Fasting blood sugar	116 mg./100 ml.
Serum chloride	102 mEq./L
Carbon dioxide combining power	33.4 mM/L
Serum sodium	140 mEq./L
Serum potassium	3.8 mEq./L
Standard glucose tolerance test	Fasting, 122 mg./100 ml.; half hour, 187; one hour, 250; two hours, 267; three hours, 175 mg./100 ml.
Standard intravenous insulin tolerance test	Fasting, 116 mg./100 ml.; twenty minutes, 99; thirty minutes, 100; forty minutes, 82; sixty minutes, 92; ninety minutes, 79 mg./100 ml.
Urinary 17-ketosteroids	21.4 mg./24 hrs.
Plasma 17-hydroxycorticosteroids	42γ/100 ml.
Electrocardiogram	Left ventricular enlargement
Chest x-ray examination	Normal heart; mild osteoporosis
Benzodioxane test	Normal findings

hirsutism of the face, trunk, arms and legs was present. The ocular fundi showed arteriovenous nicking and scattered hemorrhages and exudates. The heart and lungs were normal. The abdomen was protuberant, but no striae were present. Pelvic examination was unsatisfactory because of the marked obesity. The external genitalia were normal. The skin over the extremities was thin, atrophic and semitranslucent. There was ankle edema. Table 1 summarizes the admission laboratory data.

Second admission (Oct. 15–30, 1951)

The patient was readmitted for operation. On October 18, 1951, after preoperative preparation with cortisone, an exploratory procedure was carried out through a left transthoracic-transdiaphragmatic incision. The left adrenal was seen to be markedly enlarged. Palpation of the right adrenal indicated that it was also increased in size. There was no suggestion of tumor in either gland. A large pedunculated left ovarian cyst was discovered but this was not disturbed. More than 90 per cent of the left adrenal was resected; this portion of the gland weighed 16 grams. Microscopic sections disclosed only diffuse hyperplasia. With the exception of a postoperative pleural effusion, the patient's course was uncomplicated and cortisone was discontinued on the seventh postoperative day. Following operation, the level of plasma steroids fell from an original value of 42 μg. per 100 ml. to 12 μg.—a normal figure. The eosinophil count, initially low, was now 130 to 160 per cu. mm.

Third admission (Nov. 6–16, 1951)

No significant changes in the physical findings had occurred. The previously noted left ovarian tumor was removed. It proved to be a large papillary cystadenoma with no identifiable adrenal-like tissue. The postoperative course was uneventful.

Interval: In the ensuing eight weeks, the pedal edema disappeared, the blood pressure fell to 126/80 mm. Hg and the weight decreased markedly. The level of blood steroids remained normal during this period. Thereafter the weight and blood pressure returned gradually to their former excessive values, ankle edema reappeared, eosinophils disappeared from the peripheral blood, and the plasma 17-hydroxycorticosteroid level rose to 30 μg. per 100 ml.

Fourth admission (Feb. 5–Apr. 8, 1952)

The patient was readmitted and a total right adrenalectomy was carried out on February 7, 1952. The right adrenal weighed 22.5 grams and, like the left one, was found to show only diffuse hyperplasia. Postoperatively a severe adrenal crisis occurred, with a series of clinical symptoms similar to those in all subsequent episodes of adrenal insufficiency. Gradually increasing sinus tachycardia (maximum, 160 per minute) and severe hypotension were associated with severe nausea, cold extremities, diaphoresis, and a feeling of impending doom. Treatment consisted of large amounts of cortisone acetate (400 mg. in the first twenty-four hours), 150 ml. of aqueous adrenal extract, and adequate parenteral fluid. These measures resulted in rapid improvement. Bronchopneumonia appeared forty-eight hours after operation but cleared within a week after therapy with antibiotics. During the second week following this third operation (right total adrenalectomy), cortisone dosage was gradually decreased. Persistent nausea was prominent during this period. Subsequently frank adrenal insufficiency occurred. With increased cortisone dosage and aqueous adrenal extract, these symptoms subsided. At the end of the fourth postoperative week, severe pyelonephritis developed and precipitated another episode of acute adrenal insufficiency. Efforts to control the urinary tract infection during the next three weeks were not completely successful. The initially purulent urine became normal in appearance but was found on repeated culture to contain *E. coli*. A trial of polymyxin therapy was associated with the development of an acute psychosis which subsided after discontinuance of this drug. On April 8, 1952, two months after right adrenalectomy, the patient was discharged from the hospital. Her blood pressure was 120/64 mm. Hg. She was clinically improved, and was being maintained on 50 mg. of cortisone acetate daily by mouth, 1 mg. of desoxycorticosterone acetate (DCA) sublingually three times weekly, and 4 grams of sulfadiazine daily. The week prior to

discharge from the hospital, treatment with ACTH (adrenocorticotropin) was begun in an attempt to stimulate the left adrenal remnant. During ACTH administration, the plasma 17-hydroxycorticosteroid level rose from 5 to 25 μg. per 100 ml., indicating the presence of residual functioning adrenal tissue.

Interval: Following discharge from the hospital, pain and tenderness developed in the proximal joints of the fingers and in the elbows, shoulders and knees. These symptoms, suggestive of mild rheumatoid arthritis, disappeared after three months.

Fifth admission (May 12–21, 1952)

Oxytetracycline was administered intravenously over a ten-day period in an effort to clear the chronic urinary tract infection. The urinary sediment decreased but *E. coli* still were present in the urine. During this admission, menstruation occurred for the first time in eight years.

Interval: During the next five months, progressive clinical improvement was observed. The urine cultures showed no growth on prolonged therapy with sulfadiazine. The blood pressure remained normal. The weight decreased to 160 pounds. Truncal obesity was less evident and hirsutism of the arms and legs decreased. The menses were normal. The patient was able to carry out all her household duties.

On July 1, 1952, a dose of 100 units of long-acting ACTH was administered intramuscularly. No rise in urinary 17-hydroxycorticosteroids or 17-ketosteroids occurred. This was in contrast to the rise in the blood steroid level that had resulted when ACTH was administered three months previously.

Sixth admission (Oct. 3–6, 1952)

The patient was readmitted for a final attempt to stimulate the left adrenal remnant with ACTH. The blood pressure was 125/75 mm. Hg and other vital signs were normal. The weight was 164 pounds. Numerous black freckles were present over the arms, chest and face, and there was some increase in palmar pigment. The cervicodorsal fat pad had disappeared. Laboratory studies showed that the volume of packed red cells, the leukocyte count and the differential leukocyte count were within normal limits. Results of urinalysis were normal. On October 4, an intravenous infusion, consisting of 25 units of ACTH (Armour Lot #K 23601) in 1,000 ml. of 5 per cent dextrose in water, was begun. After the patient had received 50 ml. of the solution, she complained of a feeling of oppression. Within five minutes she became nauseated and vomited. Severe facial and lingual edema developed. The blood pressure declined precipitously to 60/40 and the pulse rose to 160. Marked diaphoresis occurred and was followed by a cramping abdominal pain and a mucoid, watery stool. No epinephrine was given. The infusion was discontinued immediately. Two hours after the beginning of the reaction, generalized urticaria appeared. The blood pressure had returned to normal within three hours and she appeared better. The urticaria receded in twenty-four hours. The patient made a rapid full recovery and was discharged from the hospital.

Interval: Since the last admission, the patient has been maintained on 37.5 mg. of cortisone acetate per day. DCA was discontinued because of recurrent edema. The patient remains normotensive, with stable weight, normal eye grounds, and a continued feeling of well being.

CLINICAL SUMMARY

A 40-year-old housewife who had severe hypertension for seven years, entered the hospital with most of the characteristic findings of Cushing's

syndrome. After undergoing subtotal adrenalectomy, the patient had a postoperative course complicated by repeated adrenal crises, marked vasomotor instability, bronchopneumonia, severe pyelonephritis and an acute psychosis which presumably resulted from polymyxin therapy. There was a severe anaphylactic reaction to an infusion of ACTH. Two years after operation, the patient is receiving maintenance cortisone therapy and has no signs or symptoms of recurrence of Cushing's syndrome.

IMMUNOLOGIC STUDIES

Serum withdrawn from the patient seventy-two hours after the anaphylactic reaction was used to carry out Prausnitz-Küstner passive transfer tests. Two donors were employed and four ACTH preparations were examined. The results are given in Table 2. Markedly positive results with transfer tests were obtained with all ACTH preparations except with a highly purified pork product supplied by Dr. E. B. Astwood. Both pig and sheep preparations gave positive results. Thus the reaction could not be attributed to species specificity. Precipitin tests, using the same serum from the patient together with the various ACTH preparations, gave negative results. To study the problem further, two 200-gram virgin female guinea pigs were each given 2 ml. of the patient's serum by the intravenous route. Forty-eight hours later, the guinea pig uteri were used in the Dale-Schultz procedure[2] and ACTH preparations were added to the bath in a final concentration of 50 micrograms per ml. No contractions occurred with these ACTH preparations, although they had been shown capable of reacting in normal subjects with the patient's serum to give positive results with the Prausnitz-Küstner test. The patient's serum, after being heated to 56° C. for thirty minutes, proved incapable of giving a positive skin reaction in passive transfer tests.

The serum withdrawn from the patient six months and sixteen months after the original anaphylactic reaction, still gave positive results with skin tests (passive transfers). At the latter time (sixteen months after the original anaphylactic reaction) Armour's lyophilized ACTH (Lot #J 19807) and a more recently developed preparation (Lot #L 57704) gave positive reactions in passive transfer skin tests. An even more strongly positive response with the skin test was obtained with a preparation of whale ACTH (Nyegaard & Co., Lot # 04617033). The strongest reaction on passive transfer was observed with a preparation of equine anterior pituitary extract (Victory Packing Co. Ex-1B).

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[2] We are grateful to Dr. Arthur French and to Dr. Richard Nelson for their assistance in performing the Dale-Schultz test.

TABLE 2. RESULTS OF PRAUSNITZ-KÜSTNER PASSIVE TRANSFER TESTS

Test substance	Species	Time after inj. (mins.)	Erythema (mm. largest diam.)				Wheal (mm. largest diam.)			
			Control		Test site		Control		Test site	
			A	B	A	B	A	B	A	B
ACTH, Armour lot #K23601	Pork	15	50	30	70	45	14	17	35	38
		60	45	30	55	40	14	12	47	30
ACTH, Armour lot #53409	Pork	15	55	25	65	40	13	14	45	30
		60	30	28	70	35	16	21	50	28
ACTH, Li lot #L2397BP	Sheep	15	40	25	60	40	0	11	0	10
		60	40	20	65	35	0	11	0	10
ACTH, Astwood oxycellulose		15	40	12	35	0	7	11	7	10
		60	25	0	25	0	4	12	12	10
Saline			0	0	0	0	0	0	0	0

Two normal subjects, A and B, received intracutaneously 0.1 ml. of the patient's serum. Twenty-four hours later, ACTH was put into same site and into a control site on the opposite arm. Both the erythema and the wheals were significantly larger at the site of previous serum injection than at the control site with all but one ACTH preparation.

DISCUSSION

The signs and symptoms of hypercorticism (Cushing's syndrome) include muscle wasting, abnormal fat distribution with thin extremities, hirsutism, hypertension, osteoporosis, diabetes, ecchymoses, amenorrhea in females, a cervico-thoracic fat pad, full facies, muscle weakness, thin skin, and purple striae (5). Our patient differed significantly from this pattern in only two respects. First, hypertension of the extreme degree manifest in our patient is unusual and led us to take particular care, before operation, to exclude all other known causes of increased arterial tension. Secondly, violaceous striae were not present.

The characteristic laboratory findings of Cushing's syndrome are well demonstrated in this patient (Table 1). Leukocytosis, eosinopenia, hyperglycemia with a diabetic glucose tolerance curve, a "resistant" type of insulin tolerance curve, increased serum carbon dioxide combining power, and abnormal urinary and plasma steroid levels are well known manifestations (6) (7). The moderately increased value for urinary ketosteroids in this patient suggested the preoperative diagnosis of adrenal hyperplasia,

rather than adrenal malignancy. Forbes and Albright (8) have pointed out that extremely high urinary 17-ketosteroid excretions are almost invariably associated with adrenal carcinoma, whereas only moderately elevated values are present in adrenal hyperplasia. The determination of the level of circulating 17-hydroxycorticosteroids in the plasma affords a sensitive index of the status of adrenal function (7). This procedure proved valuable in our patient in that the initial elevated value for 17-hydroxycorticosteroids fell to a normal level after removal of the major portion of the left adrenal gland and then rose again to an abnormal height when the Cushing's syndrome reappeared. Moreover, it proved to be a useful method for ascertaining whether or not the adrenal remnant remaining after subtotal adrenalectomy was responsive to ACTH.

The preoperative and operative management of our patient was carried out in the manner usually employed when extirpation of the adrenals is used for the treatment of Cushing's syndrome (2). Large doses of cortisone were given intramuscularly preoperatively, and cortisone in solution was administered slowly by the intravenous route throughout the operation.

It is well known that the postoperative course following subtotal or total adrenalectomy in Cushing's syndrome is likely to be difficult (2) (9). This patient was no exception. In the period immediately following operation, hypotension, tachycardia, diaphoresis, nausea and vomiting developed, in spite of the preoperative and postoperative cortisone therapy. Others have observed similar findings, all of which suggest acute adrenal insufficiency. The occurrence of a rising pulse rate provides useful clinical evidence of the imminence of this syndrome, and was a helpful sign in the patient described here. The fact that such episodes in our patient and in other similar patients respond to administration of cortisone and desoxycorticosterone acetate, leaves little doubt that these represent true adrenal insufficiency. The acute crises were not attended by hypoglycemia or dehydration.

Postoperatively irritability and mental depression developed in this patient, persisting for many weeks. Similar psychiatric difficulties occur in spontaneous Addison's disease (10) and in Cushing's syndrome (2, 9, 11) before and after operation.

In the case under discussion, anorexia, nausea, vomiting and even coma were observed as long as seven weeks postoperatively. Others have noted similar findings as late as the third to fourth week after operation and have referred to the late complications as "delayed reactions" (2, 9). There is no certain explanation of these events. In our case, the "delayed reaction" like the immediate postoperative distress, responded to increased cortisone dosage.

It is of considerable interest that this patient had symptoms similar to those of mild rheumatoid arthritis during the fourth to the sixth month fol-

TABLE 3. SUMMARY OF REPORTS OF HYPERSENSITIVITY TO ACTH

Authors & reference	No. of patients	Source of ACTH (species)	Type of reaction	Remarks
Traeger (12)	4	Pork	Urticaria 2, drug fever 1, anaphylaxis 1	
Markson (13)	1	Pork	Urticaria	
Stillman & Bayles (14)	1	Pork	Not specified	
Brown & Hollander (15)	6	Pork	Urticaria 5, anaphylactic shock 1	Beef ACTH substituted without difficulty.
Stevenson (16)	6	Pork	Urticaria 3, anaphylaxis 2, chest pain 1	Direct intracutaneous tests with ACTH gave positive results.
Hill (17)	1 several	Beef	Urticaria	Use of antihistaminics allowed continued admin. of the ACTH.
Sauer (18)	1	Not specified	Erythematous dermatitis	Complement fixing antibodies demonst in serum. Passive transfer & intradermal tests gave negative results.
Driscoll & Sass (19)	1	Not specified	Anaphylactic shock	Novocaine also in material admin. Direct dermal tests for both gave positive findings.
Feinberg *et al.* (20)	1	Pork	Urticaria; anaphylaxis	Passive transfer tests gave positive reaction to pork ACTH only. Direct intradermal test gave positive reaction to beef & sheep ACTH, sheep peptide ACTH, Pitocin, Pitressin & Antuitrin.
Zeligman (22)	1	Not specifided	Contact dermatitis	
McCombs *et al.* (23)	several	Pork	Local urticaria at injection site	
Segal & Herschfus (24)	1	Pork	Anaphylaxis	
Evans & Rackemann (25)	1	Not specified	Local urticaria at injection site	
Forsham (26)	3	Pork	Anaphylactoid reaction 2, urticaria 1	
West (27)	1	Not specified	Generalized urticaria	
Ufford (28)	1	Not specified	Fatal anaphylaxis	Received also Euphyllin i.v.
Ufford (28)	1	Not specified	Generalized urticaria	
Roskam *et al.* (29)	1	Not specified	Serum sickness	Positive reaction to passive transfer with ACTH.
Swift (30)	1	Not specified	Anaphylaxis	Direct and passive transfer skin tests gave positive results for beef and pork ACTH and for anterior pituitary extract, but negative results with Pitocin.

lowing the second adrenalectomy. These appeared when the patient was receiving cortisone in doses which sufficed to prevent manifestations of adrenal hypofunction. The joint pains and stiffness bore a striking resemblance to those reported by Nichols and coworkers (9) and mentioned by others (2). We have observed this phenomenon in one other patient following radical subtotal adrenalectomy for Cushing's syndrome.

Undoubtedly the most unusual feature of this case was the occurrence of a severe anaphylactic response to ACTH which was being administered intravenously, eight months after the last operation. This was a classic anaphylactic reaction with hypotension, sweating, abdominal pain, diar-

rhea, edema and urticaria. The patient's serum appeared to contain a reagin-type antibody, since it produced positive responses with Prausnitz-Küstner tests in donors unless it was preheated to 56° C. for thirty minutes. Moreover, precipitin tests and the Dale-Schultz test gave negative reactions with the patient's serum. Pig, sheep, horse and whale ACTH preparations gave positive results with passive transfer tests. It cannot be stated whether the antibody was one formed to ACTH or to an impurity in the ACTH. The negative transfer result with the oxycellulose pork ACTH can mean either that the oxycellulose method of preparation modifies the antigenic character of the ACTH or that the antibody was formed in response to some trace material other than the ACTH.

A tabulation of some of the previously described hypersenstivity reactions to ACTH is presented in Table 3. It will be noted that generalized urticaria and anaphylactic shock are the usual manifestations. In at least 1 instance, a fatal anaphylactic reaction was observed (28). In 1 patient, an illness resembling serum sickness appears to have resulted from ACTH administration (29).

In individuals with sensitization to ACTH, the results of skin tests (direct or passive transfer) often are found to be positive. The positive reactions may be restricted to ACTH preparations from a particular species or they may extend to preparations from many species. The latter situation was present in the patient presented in this report. This patient was found to retain the passively transferable antibodies for at least sixteen months after her anaphylactic response to ACTH.

SUMMARY

A detailed case report is presented of a 40-year-old housewife who underwent subtotal adrenalectomy for Cushing's syndrome. The difficulties arising in the postoperative period included recurrent crises of adrenal insufficiency, infections, mental depression and an episode suggesting mild rheumatoid arthritis.

An unusual feature was the occurrence of an acute anaphylactic reaction to ACTH in the eighth postoperative month. The patient's serum was observed to contain reagin-like antibodies to several ACTH preparations, including samples derived from sheep, pig, horse and whale pituitaries.

A brief discussion of the significance of the findings in this patient is presented.

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Editorial

CURRENT THERAPY OF VIRILIZING ADRENAL HYPERPLASIA

IN YEARS past the diagnosis of adrenal hyperplasia in a girl with virilism meant a sentence to a life of frustration and humiliation. There seemed to be no way to give these unfortunate girls the chance of a normal life. As is well known, the best therapy at that time involved attempted plastic alteration of the external genitalia. At best, this still left the patient a hirsute, sterile female. The general availability of cortical hormones and their proper application to the therapy of adrenal hyperplasia, as introduced by Wilkins *et al.* in 1950 (1), have opened new vistas of normalcy to these patients.

In the teaching clinic published in the January issue of this journal (2) it was shown how the diagnosis can be established. These female patients are either pseudohermaphroditic infants or virilized children, adolescents or adults, depending upon their age at the onset of the disease. They uniformly show both elevated 17-ketosteroid and pregnanediol chromogen excretion in the urine. To establish the diagnosis, particular emphasis should be placed on the effect of intravenous administration of compound F or of intramuscular administration of cortisone upon the patient's 17-ketosteroid and pregnanediol chromogen excretion (3).

Once the physician has established the diagnosis, he should pause for a moment to consider the fundamental lesion present and institute therapy accordingly. We agree with Jailer (4) that in this disease the adrenal is incapable of adequate hydroxylation of the 11 and 21 positions of a C-21 steroid. This leads to over-production by the adrenal of an incomplete cortical steroid, 17-hydroxyprogesterone, instead of the normal production of 17-hydroxycorticosterone (compound F). It is known that 17-hydroxyprogesterone is androgenic and that its oral administration in man leads to increased excretion of 17-ketosteroids (4). It is our belief also that metabolism of the 17-hydroxyprogesterone results in urinary excretion of the pregnanetriol characteristic of this disease (5). In the usual colorimetric determinations of pregnanediol, the pregnanetriol produces a color much like that of pregnanediol, and the pregnanediol chromogen in the urine in this disease results from both pregnanediol and pregnanetriol (6). The 17-hydroxyprogesterone is thought to be incapable of inhibiting hypophyseal production of ACTH. There is therefore an increased production of ACTH

(7), with consequent hyperplasia of the adrenal cortex and further hypersecretion of the offending steroid. Since compound F and cortisone are natural steroids capable of inhibiting the hypophyseal production of ACTH, they may break this vicious cycle and thereby remove the stimulus for the hyperplasia. The production of the abnormal masculinizing 17-hydroxyprogesterone decreases to inconsequential levels, as a result of which there is a concomitant decrease in 17-ketosteroids and the disappearance of pregnanediol chromogen from the urine.

It is interesting that although Wilkins *et al.* have shown both oral and intramuscular cortisone to be effective therapy (8), adrenal hyperplasia is one of the few conditions in which parenteral administration of cortisone is preferable to oral administration. This is undoubtedly due to the fact that inhibition of the pituitary is more evenly sustained by slow absorption from the intramuscular deposit. The oral route, with its rapid absorption and excretion, inhibits the pituitary in a seesaw fashion, or fails to inhibit it, so that the larger amounts needed for inhibition will often lead to the development of iatrogenic Cushing's syndrome. On the other hand, in these same patients, by administration of smaller amounts parenterally, it has been possible to inhibit the pituitary sufficiently to cause remission of the disease without development of Cushing's syndrome. Since patients differ in their sensitivity to cortisone, the amounts required vary widely from patient to patient. Some patients require as little as 12.5 mg. per day, others as much as 100 mg. per day parenterally. In the adequately treated patient resumption of the normal menstrual cycle, associated with mammary development, disappearance of acne and oiliness of the skin, and slight decrease in hirsutism should be expected. In our experience, satisfactory clinical remission has always been seen and has been associated with decreased excretion of the 17-ketosteroid and pregnanediol chromogen, provided therapy has been adequate.

At present, this treatment does not appear to be curative of the underlying pathologic condition and must be continued indefinitely. Most patients require intramuscular cortisone acetate therapy at least every third day. Administration of larger amounts at less frequent intervals does not seem to be advantageous. Therefore, there is a practical need for a form of treatment which will permit injections at less frequent intervals, or oral administration, without the danger of the development of Cushing's syndrome.

This ideal agent must fulfill certain requirements. The successfully treated patient will be deprived of her already inadequate supply of normal adrenocortical hormones, since ACTH production has been completely inhibited. Therefore, the ideal agent must have sufficient adrenocortical activity to maintain life and health on the dosage employed, just as if the

patient had no adrenal glands. If in this agent the ability to inhibit pituitary ACTH production should preponderate over the ability to produce Cushing's syndrome, this would permit possible oral administration with greater ease and efficacy. Of equal value would be a compound administered parenterally which would reach the inhibiting threshold without producing hyperadrenocorticism, and would maintain pituitary inhibition for several months following a single administration.

We have found weekly intramuscular injections of compound F acetate to be an improvement over the intramuscular injection of cortisone acetate daily or every other day. The same degree of depression of the urinary 17-ketosteroid and pregnanediol chromogen excretion can be obtained, whether 100 mg. of cortisone be given intramuscularly daily or 250 mg. of compound F acetate be given intramuscularly once a week. Whether or not the compound F acetate injection can be given at less frequent intervals and still be adequate for maintenance of clinical remission is not yet known. We believe this represents an advance in the therapy of adrenal hyperplasia with virilism and deserves further investigation.

It is important to remember that in these adequately treated patients there is an inhibited hypophyseal production of ACTH and probably an atrophic adrenal. Therefore, they have little or no ability to respond to stress with the increased pituitary-adrenal axis function seen in the normal response to stress. Thus, if an infection develops or a surgical procedure becomes necessary, these patients must be treated as if they had no adrenals. This situation is well known in patients with normal adrenals who are receiving cortisone for other reasons. So far as we know, no instances of adrenal failure due to this condition have been reported in treated patients with adrenal hyperplasia. However, it is a potential danger which must be anticipated.

The practice of medicine offers few satisfactions which can compare with conversion of a masculinized girl, who has been told that the prognosis is hopeless, to an essentially normal woman capable of the fulfillment of motherhood.

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Letters to the Editor

THE QUESTION OF THE DESTRUCTION OF 17,21-HYDROXYCORTICOSTEROIDS BY PHOSGENE

To the Editor:

In several laboratories, the Nelson and Samuels method (1) for the determination of 17,21-hydroxycorticosteroids in blood plasma has not yielded reproducible analytical results. Eik-Nes, Nelson and Samuels (2) have suggested the possibility that destruction of these corticosteroids by phosgene generated in chloroform which has been exposed to either direct sunlight or fluorescent light, may be an important factor in these analytical difficulties.

In order to test this hypothesis, we saturated chloroform solutions of hydrocortisone of varying concentrations with a stream of phosgene gas for sixty minutes at 25° C. Evaporation of the chloroform was effected by a stream of clean, dry air in the hood. The residue was dissolved in butanol, and the Porter-Silber method was applied to 1-milliliter portions, according to the modification of Reddy, Jenkins and Thorn (3). In addition 17,21-hydroxycorticosteroid values were determined chemically on human urine specimens of varying corticosteroid concentrations, by the method of Glenn and Nelson (4), employing beta-glucuronidase hydrolysis of urine and chromatography on Florisil. After chromatography, the solvent was evaporated. The dry urinary extracts were dissolved in 15 milliliters of chloroform and were exposed to a stream of phosgene gas for sixty minutes at 25° C. These solutions were then treated in a manner identical with that described for the hydrocortisone solutions. Duplicate control samples of both hydrocortisone and urinary extracts, not exposed to phosgene, were otherwise treated identically.

The results in Table 1 indicate that subjecting varying concentrations of hydrocortisone to a high degree of exposure to phosgene did not result in the loss of the ability of hydrocortisone to yield chromogenic products, measurable at 410 mμ, when incubated with phenylhydrazine in concentrated sulfuric acid. A similar conclusion may be drawn from Table 2 regarding urinary corticosteroids.

In the oxidation of chloroform by oxygen in the presence of ultraviolet light, phosgene is but one of a number of possible compounds found. Among

TABLE 1. THE EFFECT OF PHOSGENE ON CHLOROFORM SOLUTIONS OF HYDROCORTISONE

Hydrocortisone conc. (γ/ml.)	Corrected optical density*	
	Untreated	Treated with phosgene
2	.010	.011
5	.029	.029
10	.058	.059
25	.148	.146
50	.284	.288
100	.490	.482

* Coleman Junior spectrophotometer reading at 410 mμ, corrected for reagent blank.

products which may be formed are HCl, Cl_3COOH, and many free radicals. The mechanism is not known as yet, but the oxidation could take place by a light-catalyzed free radical reaction. Phosgene, however, is the primary product formed, and the foregoing data indicate that phosgene is not the cause of loss of steroid from chloroform solutions.

It should be emphasized that commercial chloroform of Reagent or U.S.P. grades contains about 0.75 per cent alcohol, which should suffice to inactivate any phosgene which may be formed or to inhibit effectively possible auto-oxidation. This leads us to doubt that phosgene or auto-oxidation products of chloroform may be the cause of the lack of reproducibility of the Nelson-Samuels method reported in the literature. In our work we redistilled U.S.P. grade chloroform twice over sodium carbonate,

TABLE 2. THE EFFECT OF PHOSGENE ON FLORISIL-PURIFIED CHLOROFORM URINARY EXTRACTS

Patient	Urinary corticosteroids (mg./24 hrs.)	
	Untreated extract	Extract treated with phosgene
F.H.	0.61	0.61
J.E.	2.25	2.34
P.O.	5.50	5.52
W.K.	7.41	7.35
D.R.	11.29	11.56
P.H.	14.63	14.67
N.G.	17.84	17.97
D.O.	38.03	37.89

using a 40-inch glass-bead packed column. This chloroform was then stored as long as three weeks without showing any effect when used in 17, 21-dihydroxycorticosteroid standard samples. This treatment may serve to remove some steroid-destroying impurity present in commercial chloroform.

Further investigations on the effect of chloroform oxidation products on steroids giving the Porter-Silber and blue tetrazolium reactions are being undertaken with the aid of infra-red analysis.

Acknowledgment

We are indebted to Dr. Elmer Alpert of Merck & Company for generous supplies of compound F, and to Drs. J. Phillip Mason and George R. Thomas, Graduate School, Boston University, for generously making available to us the phosgene gas.

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THE PRESENCE OF Δ^4-ANDROSTENE-3,17-DIONE IN PRENATAL AND POSTNATAL HUMAN ADRENAL GLANDS*

TO THE EDITOR:

The development of paper partition chromatographic methods sufficiently sensitive to permit the detection and characterization of minute

* This work was supported in part by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, Contract No. DA-49-007-MD-418, and by contract AT(30-1)-918, U. S. Atomic Energy Commission.

TABLE 1. GROSS AND HISTOLOGIC OBSERVATIONS ON 2 IMMATURE AND 1 MATURE ADRENAL GLAND

Specimen	Sex	Age	Wt. of adrenal (Gm.)	Observations	
				Gross	Histologic
A-52-79	F	31 wks. gestation	2.8 2.7		Narrow permanent cortex, chiefly fetal.
A-52-80	F	40 wks. gestation	5.1 4.3	Enlarged adrenals. Multiple congenital anomalies (polycystic kidneys, horseshoe, kidney, microgyria, pulmonary hypoplasia).	Large lipid deposits in adrenals; very small fasciculata.
A-52-78	M (hermaphrodite)	Died 5 min. after birth	4.0 3.5	Multiple congenital abnormalities (absence of penis, one hypoplastic testis, some remnant of external female genitalia, marked renal hyperplasia). Marked hyperplastic adrenals with gyriform external shape.	Hyperplastic, fetal cortex with large fat deposits; marked reduction of definitive cortex.

amounts of steroids appears to offer great promise in the study of hormones produced by the fetal and postnatal human adrenal. The present paper is concerned with a study of C_{19} steroids in immature and mature adrenal glands from 2 female fetuses and from a newborn male pseudohermaphrodite. The gross and histologic findings on each specimen are summarized in Table 1.

Both adrenal glands from each subject were homogenized with distilled water in a Waring blendor at 4° C. The homogenates were extracted four times with hot acetone, and the extract partitioned between pentane and 70 per cent methanol. The fat-free extracts (methanol fractions) were dissolved in redistilled ethyl acetate, washed with 10 per cent NaOH and distilled water, and taken to dryness. The residues were next placed on filter-paper strips and chromatographed twice in the solvent system heptane-propylene glycol (1).

The chromatograms of the extracts from one fetus and from the hyperplastic adrenals contained one zone each which reacted with the Zimmermann and 2,4-dinitrophenylhydrazine reagents, and absorbed ultraviolet light (1) (Table 2). The mobility of the reactive material on the chromatograms was similar to that of a standard sample of Δ^4-androstene-3,17-dione. Chromic acid oxidation (2) of the eluates of the reactive zones followed by chromatography of the oxidation products yielded only one zone which was identical in its color reactions and mobility with Δ^4-androstene-3,17-dione. Reacting the eluates with Girard reagent T and chromatography of the steroid-hydrazones formed (3) showed the presence of a steroid-dihydrazone without a free polar group (hydroxyl or nonreactive carbonyl

TABLE 2. R_T VALUES* AND COLOR REACTIONS OF THE C_{19} STEROID FOUND ON CHROMATOGRAMS OF ADRENAL EXTRACTS FROM 2 NORMAL FEMALE FETUSES AND FROM 1 NEWBORN MALE PSEUDOHERMAPHRODITE WITH ADRENAL HYPERPLASIA

Type of material	Free steroid (ligroin-propylene glycol)				After CrO_3 oxidation (ligroin-propylene glycol)			Girard reagent T hydrazone (butanol-H_2O) (84:16)
	Ultraviolet absorption	Color reaction: Zimmermann	Color reaction: DNPH†	R_T	Ultraviolet absorption	Color reaction: Zimmermann	R_T	Mobility (cm./hr.)
Fetal adrenals 1. Normal immature	+	+ (purple)	+ (orange)	1.76	+	+ (purple)	1.76	0.14
2. Normal	−	absent		−	−	−	−	−
Hyperplastic adrenals	+	+ (purple)	+ (orange)	1.65	+	+ (purple)	1.76	0.14
Δ^4-Androstene-3,17-dione (reference standard)	+	+ (purple)	+ (orange)	1.70	+	+ (purple)	1.70	0.15

* R_T = ratio of mobility of steroid to mobility of androsterone (=1.00 cm./hr.).
† DNPH = 2,4-dinitrophenylhydrazine in 1N HCl.

group). The mobility of the dihydrazone was identical with that of Δ^4-androstene-3,17-dihydrazone.

No evidence of C_{19} steroids was found on the chromatogram of the extract of the adrenals from the second fetus.

The results indicate the presence of the C_{19} steroid Δ^4-androstene-3,17-dione in the adrenals from one fetus and from the newborn male pseudohermaphrodite. These observations agree with, and extend the findings of the presence of Δ^4-androstene-3,17-dione in adrenal extracts (4) and perfusates (5).

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THE ISOLATION OF Δ^4-ANDROSTENE-11β-OL-3,17-DIONE FROM HUMAN ADRENAL INCUBATES*

TO THE EDITOR:

Recently several investigators have indicated that adrenal secretions may contain Δ^4-androstene-11β-ol-3,17-dione. Jeanloz (1) isolated this material after perfusion of Δ^4-androstene-3,17-dione through bovine adrenals. Hayano (2) obtained the compound after incubation of beef adrenal slices with Δ^4-androstene-3,17-dione as substrate. Romanoff (3) has reported indications of its presence in adrenal venous blood of humans. Wettstein (4) has recently mentioned the isolation of this material from hog adrenals. The present communication demonstrates that Δ^4-androstene-11β-ol-3,17-dione is a product of human adrenal incubates. Addition of corticotropin causes an increase in production of this steroid. Preliminary studies have shown that this material is second only to hydrocortisone in the quantity produced by incubation of human adrenal tissue.

In this laboratory, human adrenals removed because of severe hypertension or metastatic malignancy were sliced and incubated in plasma taken from the same patient. The procedures used for incubation and extraction of steroids from these incubates have been described in detail elsewhere (5). The crude extract was first subjected to paper partition chromatography in the toluene-propylene glycol system. This separated the faster moving 17-keto compounds from the more polar cortical products. Paper chromatography of the faster moving substances was then carried out in the methylcyclohexane-propylene glycol system.

In the fraction in which Δ^4-androstene-11β-ol-3,17-dione should appear, large quantities of material absorbing ultraviolet light at 245 mμ were separated. This area was eluted with methanol, and an aliquot was rechromatographed in the methylcyclohexane-propylene glycol system on

* This work was supported in part by an Institutional Grant of the American Cancer Society and by research grant H-922C$_3$, from the National Heart Institute of the National Institutes of Health, Public Health Service.

paper with a known steroid. The isolated substance showed a mobility that agreed with the rate of movement of Δ^4-androstene 11β-ol-3,17-dione under identical conditions. The substance did not form an acetate in pyridine and acetic anhydride. On the paper strip, the material showed the Zimmermann reaction (6). An aliquot of the eluted material gave a quantitative Zimmermann value agreeing with the value obtained by determination of the density of absorption of ultraviolet light. Final purification was achieved by chromatography on a Celite-silica gel (1:1) column. In concentrated sulfuric acid, the substance showed a pale yellow color with a green fluorescence (7). The absorption spectrum of the isolated substance in concentrated sulfuric acid (8) showed peaks at 280, 380, 400 and 460 mμ in agreement with the results of the same determination on authentic Δ^4-androstene-11-β-ol-3,17dione(9). After crystallization from acetone-methylcyclohexane, the substance had a melting point of 194°–196°, which was not depressed on admixture with authentic material. Infrared spectrometry, performed through the courtesy of Dr. Thomas F. Gallagher of the Sloan-Kettering Institute for Cancer Research, Memorial Center, New York, substantiated the identification.

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ON THE TOXICITY OF 9α-FLUOROHYDROCORTISONE AND 9α-CHLOROHYDROCORTISONE

To the Editor:

The discovery (1, 2) that 9 α-halogen-substitution greatly increases both the mineralo-corticoid and the gluco-corticoid potency of cortisone and hydrocortisone stimulated numerous laboratory and clinical studies with these compounds (for literature, *cf.* (3)). Hence their toxicity is of timely interest and we should like to report briefly upon experiments in rats, designed to compare the nephrotoxic and hypertensive effects of fluorohydrocortisone and chlorohydrocortisone with those of desoxycorticosterone.

Four groups, each consisting of 10 female Sprague-Dawley rats weighing 45–54 Gm. (average 50 Gm.) at the beginning of the experiment, were sensitized to the nephrotoxic effect of corticoids by unilateral nephrectomy and the administration of 1 per cent sodium chloride as a drinking fluid. The first group served as a control, and the other three groups were treated respectively with the acetates of desoxycorticosterone (DCA 200 μg./day), 9 α-fluorohydrocortisone (200 μg./day) and 9 α-chlorohydrocortisone (20 μg./day). The daily dose of all compounds was given subcutaneously in 0.1 ml. of sesame oil. Blood-pressures of the rats were taken (tail-cuff method) on the twentieth and thirtieth days of treatment and they were sacrificed on the thirty-first day.

Under these conditions and at the dose-levels used, both fluorohydrocortisone and chlorohydrocortisone produced *nephrosclerotic* and *nephritic* changes, similar to those usually obtained by DCA (Fig. 1). These were accompanied by a *rise in blood-pressure* (from the normal of 130 mm. Hg to means of 142–170 in the three experimental groups).

Macroscopically visible nodules of *mesenteric periarteritis nodosa* occurred in the rats treated with DCA or chlorohydrocortisone, although these showed no noteworthy involution of the lymphatic organs (thymus, lymph nodes and spleen) or adrenals. On the other hand, the rats treated

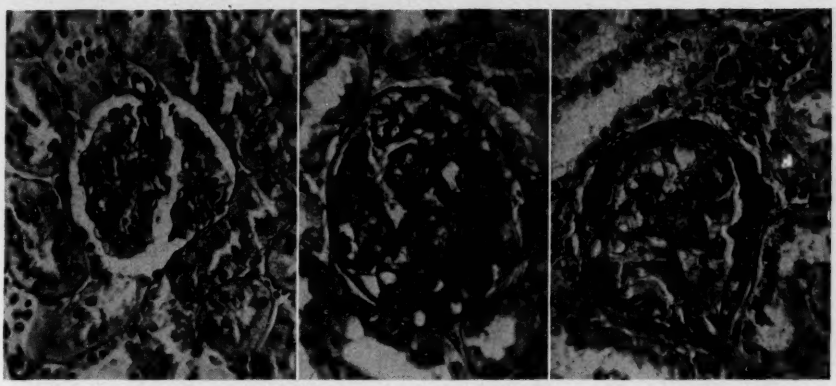

FIG. 1. *Glomerular changes following overdosage with 9-halo-hydrocortisone derivatives.* Normal control (left). Widespread destruction of the glomerular tuft with hyalin deposition in a rat treated with 9α-fluorohydrocortisone (middle) and with 9α-chlorohydrocortisone (right).

with fluorohydrocortisone exhibited no evidence of mesenteric periarteritis nodosa, but pronounced *involution of the lymphatic organs and adrenals.*

Thus, in all these respects, under our conditions of experimentation, chlorohydrocortisone resembled DCA, except that it produced manifestations of intoxication at a considerably lower dose-level. On the other hand, fluorohydrocortisone produced such morphologic changes as are usually obtained with mixtures of DCA and hydrocortisone given at high dose-levels, that is, particularly pronounced renal damage, hypertension, adrenal atrophy and involution of the lymphatic organs but no mesenteric periarteritis (4).

A detailed report of these experiments, with a review of the entire literature on 9-halo-corticoids, appears elsewhere (3). Here, we merely wish to call attention to the nephrotoxic and vasopressor effects of these compounds in rats under certain experimental conditions.

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3. SELYE, H., and HEUSER, G.: Fourth Annual Report on Stress. Montreal, Acta Inc., 1954.
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The Endocrine Society

THE 1955 ANNUAL MEETING

The Thirty-seventh Annual Meeting of The Endocrine Society will be held in the Chalfonte-Haddon Hall Hotel, Atlantic City, New Jersey, on Thursday, Friday and Saturday, June 2, 3 and 4, 1955.

Dr. Matthew Molitch, 705 Pacific Avenue, Atlantic City, New Jersey, is in charge of the local arrangements for the meeting.

All Scientific Sessions will be held in the Chalfonte-Haddon Hall Hotel. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The annual dinner is scheduled for Friday, June 3, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make hotel reservations immediately, as the hotels expect to be filled to capacity. Correspond directly with Chalfonte-Haddon Hall, advising time of arrival and departure. Make your reservations now and avoid disappointment.

THE 1955 AWARDS AND FELLOWSHIPS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers;

1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship was established in 1947. It is designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. The Fellowship is awarded on alternate years (1955, 1957, etc.) and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst, McKenna and Harrison Fellowship may be made by any member of the Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than November 1 each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by November 1 each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

The American Goiter Association

THE 1955 ANNUAL MEETING

The next annual meeting of the American Goiter Association will be held in Oklahoma City, Oklahoma, at the Skirvin Hotel, April 28, 29 and 30, 1955.

HARVARD MEDICAL SCHOOL—POSTGRADUATE COURSES IN PEDIATRICS AND PEDIATRIC ENDOCRINOLOGY

Two postgraduate courses in Pediatrics are being offered by the Department of Pediatrics, Harvard Medical School, at the Massachusetts General Hospital, Boston, in May 1955. The first (May 16 through May 24) will deal with general pediatric problems. The second (May 25 through May 27) will deal with endocrine and metabolic disorders in children and adolescents. Enrollment may be for either course or the total combined course. Those interested should write: Courses for Graduates, Harvard Medical School, 25 Shattuck Street, Boston, Mass.